

CARBOXYLIC ACIDS AND CARBOXYLIC ACID ISOSTERES OF
N-HETEROCYCLIC COMPOUNDS

Related Application Data

5 This application is a continuation-in-part of U.S. patent application 09/204,237 filed December 3, 1998, which is a continuation-in-part of U.S. patent application serial number 60/087,788 filed June 3, 1998.

10 BACKGROUND OF THE INVENTION

1. Field of the Invention

15 This invention relates to novel carboxylic acid and carboxylic acid isosteres of N-heterocyclic compounds, their preparation, their inclusion in pharmaceutical compositions, and their preparation and use for preventing and/or treating neurological disorders; for treating alopecia and promoting hair growth; for treating vision disorders and/or improving vision; for treating memory impairment and/or enhancing memory performance; and for
20 preventing and/or treating hearing loss in an animal.

2. Description of the Prior Art

25 Neurological Background

It has been found that picomolar concentrations of an immunosuppressant such as FK506 and rapamycin stimulate neurite out growth in PC12 cells and sensory nervous, namely dorsal root ganglion cells (DRGs). Lyons et al.,
30 Proc. of Natl. Acad. Sci., 1994 vol. 91, pp. 3191-3195. In whole animal experiments, FK506 has been shown to stimulate nerve regeneration following facial nerve injury and results in functional recovery in animals with sciatic nerve lesions.

Several neurotrophic factors effecting specific neuronal populations in the central nervous system have been identified. For example, it has been hypothesized that Alzheimer's disease results from a decrease or loss of nerve growth factor (NGF). It has thus been proposed to treat Alzheimer's patients with exogenous nerve growth factor or other neurotrophic proteins such as brain derived nerve factor (BDNF), glial derived nerve factor, ciliary neurotrophic factor, and neurotrophin-3 to increase the survival of degenerating neuronal populations.

Clinical application of these proteins in various neurological disease states is hampered by difficulties in the delivery and bioavailability of large proteins to nervous system targets. By contrast, immunosuppressant drugs with neurotrophic activity are relatively small and display excellent bioavailability and specificity. However, when administered chronically, immunosuppressants exhibit a number of potentially serious side effects including nephrotoxicity, such as impairment of glomerular filtration and irreversible interstitial fibrosis (Kopp et al., 1991, J. Am. Soc. Nephrol. 1:162); neurological deficits, such as involuntary tremors, or non-specific cerebral angina such as non-localized headaches (De Groen et al., 1987, N. Engl. J. Med. 317:861); and vascular hypertension with complications resulting therefrom (Kahan et al., 1989 N. Engl. J. Med. 321: 1725).

Accordingly, there is a need for small-molecule compounds which are useful for neurotrophic effects and for treating neurodegenerative disorders.

Hair Loss Background

Hair loss occurs in a variety of situations. These situations include male pattern alopecia, alopecia senilis, alopecia areata, diseases accompanied by basic skin lesions or tumors, and systematic disorders such as

nutritional disorders and internal secretion disorders. The mechanisms causing hair loss are very complicated, but in some instances can be attributed to aging, genetic disposition, the activation of male hormones, the loss of blood supply to hair follicles, and scalp abnormalities.

The immunosuppressant drugs FK506, rapamycin and cyclosporin are well known as potent T-cell specific immunosuppressants, and are effective against graft rejection after organ transplantation. It has been reported that topical, but not oral, application of FK506 (Yamamoto et al., J. Invest. Dermatol., 1994, 102, 160-164; Jiang et al., J. Invest. Dermatol. 1995, 104, 523-525) and cyclosporin (Iwabuchi et al., J. Dermatol. Sci. 1995, 9, 64-69) stimulates hair growth in a dose-dependent manner. One form of hair loss, alopecia areata, is known to be associated with autoimmune activities; hence, topically administered immunomodulatory compounds are expected to demonstrate efficacy for treating that type of hair loss. The hair growth stimulating effects of FK506 have been the subject of an international patent filing covering FK506 and structures related thereto for hair growth stimulation (Honbo et al., EP 0 423 714 A2). Honbo et al. discloses the use of relatively large tricyclic compounds, known for their immunosuppressive effects, as hair revitalizing agents.

The hair growth and revitalization effects of FK506 and related agents are disclosed in many U.S. patents (Goulet et al., U.S. Patent No. 5,258,389; Luly et al., U.S. Patent No. 5,457,111; Goulet et al., U.S. Patent No. 5,532,248; Goulet et al., U.S. Patent No. 5,189,042; and Ok et al., U.S. Patent No. 5,208,241; Rupprecht et al., U.S. Patent No. 5,284,840; Organ et al., U.S. Patent No. 5,284,877). These patents claim FK506 related compounds. Although they do not claim methods of hair revitalization, they disclose the known use of FK506 for affecting hair

growth. Similar to FK506 (and the claimed variations in the Honbo et al. patent), the compounds claimed in these patents are relatively large. Further, the cited patents relate to immunomodulatory compounds for use in autoimmune related diseases, for which FK506's efficacy is well known.

Other U.S. patents disclose the use of cyclosporin and related compounds for hair revitalization (Hauer et al., U.S. Patent No. 5,342,625; Eberle, U.S. Patent No. 5,284,826; Hewitt et al., U.S. Patent No. 4,996,193). These patents also relate to compounds useful for treating autoimmune diseases and cite the known use of cyclosporin and related immunosuppressive compounds for hair growth.

However, immunosuppressive compounds by definition suppress the immune system and also exhibit other toxic side effects. Accordingly, there is a need for small molecule compounds which are useful as hair revitalizing compounds.

Vision Disorder Background

The visual system is composed of the eyes, ocular adnexa and the visual pathways. Dysfunction of the visual system may lead to permanent or temporary visual impairment, i.e. a deviation from normal in one or more functions of the eye. Visual impairment manifests itself in various ways and includes a broad range of visual dysfunctions and disturbances. Without limitation, these dysfunctions and disturbances include partial or total loss of vision, the need for correction of visual acuity for objects near and far, loss of visual field, impaired ocular motility without diplopia (double vision), impaired or skewed color perception, limited adaptation to light and dark, diminished accommodation, metamorphopsic distortion, impaired binocular vision, paresis of accommodation, iridoplegia, entropion, ectropion,

epiphora, lagophthalmos, and scarring. See *Physicians' Desk Reference (PDR) for Ophthalmology*, 16th Edition, 6:47 (1988). The visual system may be adversely affected by various ophthalmologic disorders, diseases, injuries, and complications, including, without limitation, genetic disorders; disorders associated with aging or degenerative diseases; disorders correlating to physical injury to the eye, head, or other parts of the body resulting from external forces; disorders resulting from environmental factors; disorders resulting from a broad range of diseases; and combinations of any of the above.

The visual system is a complex system composed of numerous components. Visual impairment can involve the entire visual system, any one component, or any combination of components, depending upon the precise nature of the circumstances. The eye is composed of a lens, which is suspended in the zonules of Zinn and is focused by the ciliary body. The ciliary body also secretes aqueous humor, which fills the posterior chamber, passes through the pupil into the anterior chamber, then drains primarily via the canal of Schlemm. The iris regulates the quantity of light entering the eye by adjusting the size of its central opening, the pupil. A visual image is focused onto the retina, the fovea centralis being the retinal area of sharpest visual acuity. The conjunctiva is the mucus membrane which lines the eyelids and the eyeball, and ends abruptly at the limbus conjunctivae, the edge of the conjunctiva overlapping the cornea. The cornea is the clear, transparent anterior portion of the fibrous coat of the eye; it is important in light refraction and is covered with an epithelium that differs in many respects from the conjunctival epithelium.

The retina is the innermost, light sensitive portion of the eye, containing two types of photoreceptors, cones,

which are responsible for color vision in brighter light, and rods, which are essential for vision in dim light but do not perceive colors. After light passes through the cornea, lens system, and the vitreous humor, it enters the retina from the inside; that is, it passes through the ganglion cells and nerve fibers; the inner and outer plexiform layers, the inner and outer nuclear layers, and the internal and external limiting membranes before it finally reaches the layer of photoreceptors located near the outside of the retina, just inside the outermost pigment epithelium layer. The cells of the pigment epithelium layer act as an anatomical barrier to liquids and substances located outside of the eye, forming the "blood-retina" barrier, and provide nourishment, oxygen, a source of functionally useful substances like vitamin A, and phagocytosis of decomposition products to photoreceptor cells. There is no anatomical connection between the pigment epithelium and the photoreceptor layer, permitting separation of the layers in some pathological situations.

When rods or cones are excited by light, signals are transmitted through successive neurons in the retina itself, into the optic nerve fibers, and ultimately to the cerebral cortex. Both rods and cones contain molecules that decompose on exposure to light and, in the process, excite the nerve fibers leading from the eye. The molecule in rods is rhodopsin. The three light-sensitive molecules in cones, collectively called iodopsin, have compositions only slightly different from that of rhodopsin and are maximally excited by red, blue, or green light, respectively.

Neither rods nor cones generate action potentials. Rather, the light-induced membrane hyperpolarization generated in the outer, photosensitive segment of a rod or cone cell is transmitted from the outer segment through

the inner segment to the synaptic body by direct conduction of the electrical voltage itself, a process called electrotonic conduction. At the synaptic body, the membrane potential controls the release of an unknown transmitter molecule. In low light, rod and cone cell membranes are depolarized and the rate of transmitter release is greatest. Light-induced hyperpolarization causes a marked decrease in the release of transmitter molecules.

The transmitters released by rod and cone cells induce signals in the bipolar neurons and horizontal cells. The signals in both these cells are also transmitted by electrotonic conduction and not by action potential.

The rod bipolar neurons connect with as many as 50 rod cells, while the dwarf and diffuse bipolar cells connect with one or several cone cells. A depolarizing bipolar cell is stimulated when its connecting rods or cones are exposed to light. The release of transmitter molecules inhibits the depolarizing bipolar cell. Therefore, in the dark, when the rods and cones are secreting large quantities of transmitter molecules, the depolarizing bipolar cells are inhibited. In the light, the decrease in release of transmitter molecules from the rods and cones reduces the inhibition of the bipolar cell, allowing it to become excited. In this manner, both positive and negative signals can be transmitted through different bipolar cells from the rods and cones to the amacrine and ganglion cells.

As their name suggests, horizontal cells project horizontally in the retina, where they may synapse with rods, cones, other horizontal cells, or a combination of cells types. The function of horizontal cells is unclear, although some mechanism in the convergence of photoreceptor signaling has been postulated.

All types of bipolar cells connect with ganglion cells, which are of two primary types. A-type ganglion cells predominately connect with rod bipolar cells, while B-type ganglion cells predominately connect with dwarf and diffuse bipolar cells. It appears that A-type ganglion cells are sensitive to contrast, light intensity, and perception of movement, while B-type ganglion cells appear more concerned with color vision and visual acuity.

Like horizontal cells, the Amacrine cells horizontally synapse with several to many other cells, in this case bipolar cells, ganglion cells, and other Amacrine cells. The function of Amacrine cells is also unclear.

The axons of ganglion cells carry signals into the nerve fiber layer of the eye, where the axons converge into fibers which further converge at the optic disc, where they exit the eye as the optic nerve. The ganglion cells transmit their signals through the optic nerve fibers to the brain in the form of action potentials. These cells, even when unstimulated, transmit continuous nerve impulses at an average, baseline rate of about 5 per second. The visual signal is superimposed onto this baseline level of ganglion cell stimulation. It can be either an excitatory signal, with the number of impulses increasing above the baseline rate, or an inhibitory signal, with the number of nerve impulses decreasing below the baseline rate.

As part of the central nervous system, the eye is in some ways an extension of the brain; as such, it has a limited capacity for regeneration. This limited regeneration capacity further complicates the challenging task of improving vision, resolving dysfunction of the visual system, and/or treating or preventing ophthalmologic disorders. Many disorders of the eye, such as retinal photic injury, retinal ischemia-induced eye

injury, age-related macular degeneration, free radical-induced eye diseases, as well as numerous other disorders, are considered to be entirely untreatable. Other ophthalmologic disorders, e.g., disorders causing permanent visual impairment, are corrected only by the use of ophthalmic devices and/or surgery, with varying degrees of success.

The immunosuppressant drugs FK506, rapamycin, and cyclosporin are well known as potent T-cell specific immunosuppressants, and are effective against autoimmunity, transplant or graft rejection, inflammation, allergic responses, other autoimmune or immune-mediated diseases, and infectious diseases. It has been disclosed that application of Cyclosporin, FK-506, Rapamycin, Buspirone, Spiperone, and/or their derivatives are effective in treating some ophthalmologic disorders of these types. Several ophthalmologic disorders or vision problems are known to be associated with autoimmune and immunologically-mediated activities; hence, immunomodulatory compounds are expected to demonstrate efficacy for treating those types of ophthalmologic disorders or vision problems.

The effects of FK506, Rapamycin, and related agents in the treatment of ophthalmologic diseases are disclosed in several U.S. patents (Goulet et al., U.S. Patent No. 5,532,248; Mochizuki et al., U.S. Patent No. 5,514,686; Luly et al., U.S. Patent No. 5,457,111; Russo et al., U.S. Patent No. 5,441,937; Kulkarni, U.S. Patent No. 5,387,589; Asakura et al., U.S. Patent No. 5,368,865; Goulet et al., U.S. Patent No. 5,258,389; Armistead et al., U.S. Patent No. 5,192,773; Goulet et al., U.S. Patent No. 5,189,042; and Fehr, U.S. Patent No. 5,011,844). These patents claim FK506 or Rapamycin related compounds and disclose the known use of FK506 or Rapamycin related compounds in the treatment of ophthalmologic disorders in

association with the known immunosuppressive effects of FK506 and Rapamycin. The compounds disclosed in these patents are relatively large. Further, the cited patents relate to immunomodulatory compounds limited to treating autoimmunity or related diseases, or immunologically-mediated diseases, for which the efficacy of FK506 and Rapamycin is well known.

Other U.S. patents disclose the use of cyclosporin, Spiperone, Buspirone, their derivatives, and other immunosuppressive compounds for use in the treatment of ophthalmologic diseases (Sharpe et al., U.S. Patent No. 5,703,088; Sharpe et al., U.S. Patent No. 5,693,645; Sullivan, U.S. Patent No. 5,688,765; Sullivan, U.S. Patent No. 5,620,921; Sharpe et al., U.S. Patent No. 5,574,041; Eberle, U.S. Patent No. 5,284,826; Sharpe et al., U.S. Patent No. 5,244,902; Chiou et al., U.S. Patent Nos. 5,198,454 and 5,194,434; and Kaswan, U.S. Patent No. 4,839,342). These patents also relate to compounds useful for treating autoimmune diseases and cite the known use of cyclosporin, Spiperone, Buspirone, their derivatives, and other immunosuppressive compounds in treating ocular inflammation and other immunologically-mediated ophthalmologic diseases.

The immunosuppressive compounds disclosed in the prior art suppress the immune system, by definition, and also exhibit other toxic side effects. Accordingly, there is a need for non-immunosuppressant, small molecule compounds, and compositions and methods for use of such compounds, that are useful in improving vision; preventing, treating, and/or repairing visual impairment or dysfunction of the visual system; and preventing, treating, and/or resolving ophthalmologic disorders.

There are also a number of patents on non-immunosuppressive compounds disclosing methods of use for permitting or promoting wound healing (whether from injury

or surgery); controlling intraocular pressure (often
resulting from glaucoma); controlling neurodegenerative
eye disorders, including damage or injury to retinal
neurons, damage or injury to retinal ganglion cells, and
5 macular degeneration; stimulating neurite outgrowth;
preventing or reducing oxidative damage caused by free
radicals; and treating impaired oxygen and nutrient
supply, as well as impaired waste product removal,
resulting from low blood flow. These non-
10 immunosuppressive substances fall into one of two general
categories: naturally occurring molecules, such as
proteins, glycoproteins, peptides, hormones, and growth
factors; and synthetic molecules.

Within the group of naturally occurring non-
15 immunosuppressive molecules, several hormones, growth
factors, and signaling molecules have been patented for
use as supplements to naturally occurring quantities of
such molecules, as well as for targeting of specific cells
where the particular molecule does not naturally occur in
20 a mature individual. These patents generally claim
methods of use for reducing or preventing the symptoms of
ocular disease, or arresting or reversing vision loss.

Specifically, Louis et al., U.S. Patent Nos.
5,736,516 and 5,641,749, disclose the use of a glial cell
25 line derived neurotrophic factor (GDNF) to stop or reverse
the degeneration of retinal neurons (i.e. photoreceptors)
and retinal ganglion cells caused by glaucoma, or other
degenerative or traumatic retinal diseases or injuries.
O'Brien, et al., U.S. Patent Nos. 5,714,459 and 5,700,909,
30 disclose the use of a glycoprotein, Saposin, and its
derivatives for stimulating neurite outgrowth and
increasing myelination. To stop or reverse degeneration
of retinal neurons, LaVail et al., U.S. Patent No.
5,667,968, discloses the use of a variety of neurotrophic
35 proteins, including brain-derived neurotrophic factor,

ciliary neurotrophic factor, neurotrophin-3 or neurotrophin-4, acidic or basic fibroblast growth factors, interleukin, tumor necrosis factor- α , insulin-like growth factor-2 and other growth factors. Wong et al., U.S.

5. Patent No. 5,632,984, discloses the use of interferons, especially interferon α -2a, for treating the symptoms of macular degeneration by reducing hemorrhage and limiting neovascularization. Finally, Wallace et al., U.S. Patent No. 5,441,937, discloses the use of a lung-derived
10 neurotrophic factor (NTF) to maintain the functionality of ciliary ganglion and parasympathetic neuron cells.

A key characteristic of factors derived from specific cell lines is their localization to specific cell lines or tissues; systemic treatment with these molecules would run
5 a substantial risk of unintended, and potentially dangerous, effects in cell lines where the genes encoding these molecules are inactive. Similarly, hormones and growth factors often activate a large number of genes in many cell lines; again, non-localized application of these
10 molecules would run a substantial risk of provoking an inappropriate, and potentially dangerous, response.

Within the category of synthetic molecules, most of the patented compounds are immunosuppressive and disclose
25 uses in treating inflammatory, autoimmune, and allergic responses, as discussed above. A few others are non-immunosuppressive and claim the ability to treat cellular degeneration, and in some cases promote cellular regeneration, most often in the context of their antioxidant properties.

30 Specifically, Tso et al., U.S. Patent No. 5,527,533, discloses the use of astaxanthin, a carotenoid antioxidant, for preventing or reducing photoreceptor damage resulting from the presence of free radicals. Similarly, Babcock et al., U.S. Patent No. 5,252,319,
35 discloses the use of antioxidant aminosteroids for

treating eye disease and injury, by increasing resistance to oxidative damage. Freeman, U.S. Patent No. 5,468,752, discloses the use of the antiviral phosphonylmethoxyalkylcytosines to reduce abnormally increased intraocular pressure.

Naturally occurring hormones, growth factors, cytokines, and signaling molecules are generally multifunctional and activate many genes in diverse cell lines. The present compounds do not, thus avoiding the unexpected, and potentially dangerous, side effects of systemic use. Similarly, the present compounds also avoid the potential unexpected side effects of introducing cell line-specific molecules into other cell lines were they do not naturally occur.

Hearing Loss Background

The epithelial hair cells in the organ of Corti of the inner ear, transduce sound into neural activity, which is transmitted along the cochlear division of the eighth cranial nerve. This nerve consists of fibers from three types of neurons (Spoendlin, H. H., in Friedmann, I. Ballantyne, J., eds. "Ultrastructural Atlas of the Inner Ear", London, Butterworth, pp. 133-164, (1984)) 1) afferent neurons, which lie in the spiral ganglion and connect the cochlea to the brainstem; 2) efferent olivocochlear neurons, which originate in the superior olivary complex; and 3) autonomic adrenergic neurons, which originate in the cervical sympathetic trunk and innervate the cochlea. In the human, there are approximately 30,000 afferent cochlear neurons, with myelinated axons, each consisting of about 50 lamellae, and 4-6 μ m in diameter. This histologic structure forms the basis of uniform conduction velocity, which is an important functional feature. Throughout the length of the auditory nerve, there is a trophic arrangement of

afferent fibers, with 'basal' fibers wrapped over the centrally placed 'apical' fibers in a twisted rope-like fashion. Spoendlin (Spoendlin, H. H. in Naunton, R. F., Fernadex, C. eds., "Evoked Electrical Activity in the Auditory Nervous System", London, Academic Press, pp. 21-39, (1978)) identified two types of afferent neurons in the spiral ganglion on the basis of morphologic differences: type I cells (95%) are bipolar and have myelinated cell bodies and axons that project to the inner hair cells. Type II cells (5%) are monopolar with unmyelinated axons and project to the outer hair cells of the organ of Corti. Each inner hair cell is innervated by about 20 fibers, each of which synapses on only one cell. In contrast, each outer hair cell is innervated by approximately six fibers, and each fiber branches to supply approximately 10 cells. Within the cochlea, the fibers divide into: 1) an inner spiral group, which arises primarily ipsilaterally and synapses with the afferent neurons to the inner hair cells, and 2) a more numerous outer radial group, which arises mainly contralaterally and synapses directly with outer hair cells. There is a minimal threshold at one frequency, the characteristic or best frequency, but the threshold rises sharply for frequencies above and below this level (Pickles, J.O. in "Introduction to the Physiology of Hearing", London, Academic Press, pp. 71-106, (1982)). Single auditory nerve fibers therefore appear to behave as band-pass filters. The basilar membrane vibrates preferentially to different frequencies, at different distances along its length, and the frequency selectivity of each cochlear nerve fiber is similar to that of the inner hair cell to which the fiber is connected. Thus, each cochlear nerve fiber exhibits a tuning curve covering a different range of frequencies from its neighboring fiber (Evans, E.F. in Beagley H.A. ed., "Auditory investigation: The Scientific

and Technological basis", New York, Oxford University Pressm (1979)). By this mechanism, complex sounds are broken down into component frequencies (frequency resolution) by the filters of the inner ear.

5 Impairment anywhere along the auditory pathway, from the external auditory canal to the central nervous system, may result in hearing loss. The auditory apparatus can be subdivided into the external and middle ear, inner ear and auditory nerve and central auditory pathways. Auditory
10 information in humans is transduced from a mechanical signal to a neurally conducted electrical impulse by the action of approximately 15,000 epithelial cells (hair cells) and 30,000 first-order neurons (spiral ganglion cells) in the inner ear. All central fibers of spiral
15 ganglion neurons form synapses in the cochlear nucleus of the pontine brainstem. The number of neurons involved in hearing increases dramatically from the cochlea to the auditory brain stem and the auditory cortex. All auditory information is transduced by only 15,000 hair cells, of
20 which the so-called inner hair cells, numbering 3500, are critically important, since they form synapses with approximately 90 percent of the 30,000 primary auditory neurons. Thus, damage to a relatively few cells in the auditory periphery can lead to substantial hearing loss.
25 Hence, most causes of sensorineural loss can be ascribed to lesions in the inner ear (Nadol, J.B., New Englands Journal of Medicine, (1993), 329:1092-1102).

Hearing loss can be on the level of conductivity, sensorineural and central level. Conductive hearing loss
30 is caused by lesions involving the external or middle ear, resulting in the destruction of the normal pathway of airborne sound amplified by the tympanic membrane and the ossicles to the inner ear fluids. Sensorineural hearing loss is due to lesions of the central auditory pathways.
35 These consist of the cochlear and dorsal olivary nucleus

complex, inferior colliculi, medial geniculate bodies, auditory cortex in the temporal lobes and interconnecting afferent and efferent fiber tracts (Adams R.D. and Maurice, V., eds., in "Principles of Neurology", (1989), McGraw-Hill Information Services Company, pp. 226-246).

Trauma due to acoustic overstimulation is another leading cause of deafness. There is individual susceptibility to trauma from noise. Clinically important sensorineural hearing loss may occur in some people exposed to high-intensity noise, even below levels approved by the Occupational Safety and Health Agency (Osguthorpe, J.D., ed., Washington D.C., American Academy of Otolaryngology-Head and Neck Surgery Foundation, (1988)).

Demyelinating processes, such as multiple sclerosis, may cause sensorineural hearing loss (Noffsinger, D., et al., Acto Otolaryngol. Suppl. (Stockh.) (1972), 303:1-63). More recently, a form of immune-mediated sensorineural hearing loss has been recognized (McCabe, B.F., Ann. Otol. Rhinol. Laryngol. (1979), 88:585-9). The hearing loss is usually bilateral, is rapidly progressive (measured in weeks and months), and may or may not be associated with vestibular symptoms.

A variety of tumors, both primary and metastatic, can produce either a conductive hearing loss, or a sensorineural hearing loss, by invading the inner ear or auditory nerve (Houck, J.R., et al., Otolaryngol. Head Neck Surg. (1992), 106:92-7). A variety of degenerative disorders of unknown cause can produce sensorineural hearing loss. Meniere's syndrome (Nadol, J.B., ed., "Meniere's Disease: Pathogenesis, Pathophysiology, Diagnosis, And Treatment," Amsterdam: Kugler & Ghedini (1989)), characterized by fluctuating sensorineural hearing loss, episodic vertigo, and tinnitus, appears to be caused by a disorder of fluid homeostasis within the

inner ear, although the pathogenesis remains unknown. Sudden idiopathic sensorineural hearing loss (Wilson, W.R., et al., Arch. Otolaryngol. (1980), 106:772-6), causing moderate-to-severe sensorineural deafness, may be due to various causes, including inner ear ischemia and viral labyrinthitis.

Regardless of the cause, there exists a need to prevent or treat sensorineural hearing loss. The present invention provides such a method.

SUMMARY OF THE INVENTION

The present invention relates to the surprising discovery that N-heterocyclic compounds containing a carboxylic acid or carboxylic acid isostere moiety may be useful for treating neurological and/or neurodegenerative disorders, for treating alopecia and related hair loss disorders, for treating vision disorders and/or improving vision, for treating memory impairment and/or enhancing memory performance, and for treating sensorineural hearing loss. Accordingly, a novel class of compounds containing an acidic moiety or an isostere thereof attached to the 2-carbon of the N-heterocyclic ring is provided.

These compounds stimulate neuronal regeneration and outgrowth and as such are useful for treating neurological disorders and neurodegenerative diseases. These compounds also promote hair growth and as such are useful for treating hair loss disorders. These compounds also are useful for treating vision disorders, improving vision, treating memory impairment, enhancing memory performance, or treating hearing loss. A preferred feature of the compounds of the present invention is that they do not exert any significant immunosuppressive activity and/or are non-immunosuppressive.

A preferred embodiment of the invention is a pharmaceutical composition containing: a therapeutically

effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere compound; and a pharmaceutically suitable or acceptable carrier.

For pharmaceutical compositions directed specifically to neurotrophic medical indications, one or more additional neurotrophic factor(s) or neurotrophic agent(s) may be administered in combination with, or otherwise included in, the composition. Similarly pharmaceutical compositions directed specifically to hair loss related medical indications may also be administered in combination with an additional agent(s). Similarly pharmaceutical compositions directed specifically to vision disorder related medical indications may also be administered in combination with an additional agent(s). Similarly pharmaceutical compositions directed specifically to memory impairment related medical indications may also be administered in combination with an additional agent(s). Similarly pharmaceutical compositions directed specifically to hearing loss related medical indications may also be administered in combination with an additional agent(s).

A preferred method or use of the invention is a method of promoting neuronal regeneration and growth in mammals, comprising administering to a mammal an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere.

Another preferred method or use of the invention is a method of treating a neurological disorder in an animal, comprising administering to an animal an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere to stimulate growth of damaged peripheral nerves or to promote neuronal regeneration.

Yet another preferred method or use of the invention is a method of preventing neurodegeneration in an animal, comprising administering to an animal an effective amount

of an N-heterocyclic carboxylic acid or carboxylic acid isostere.

Yet another preferred method or use of the invention is a method of stimulating growth of damaged peripheral nerves; comprising administering to a damaged peripheral nerve an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere.

Yet another preferred method or use of the invention is a method of treating alopecia or promoting hair growth in an animal, comprising administering to an animal an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere.

Yet another preferred embodiment of this invention is a method for treating a vision disorder, improving vision, treating memory impairment, or enhancing memory performance in an animal, comprising administering to an animal an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere.

Yet another preferred embodiment of this invention is a method for treating sensorineural hearing loss in an animal, comprising administering to an animal an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere.

The present invention further contemplates a process for preparing the an N-heterocyclic carboxylic acid or carboxylic acid isostere of the invention, comprising acidifying an intermediate compound.

The present invention further contemplates the compound(s) of the invention for use in treatment of a disease. In particular, the present invention contemplates the compound(s) of the invention for use in treatment of the disorders enumerated herein.

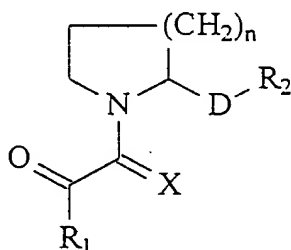
The invention further contemplates the compound(s) of the invention for use in the preparation of a medicament or pharmaceutical composition. In particular, the

invention contemplates the compound(s) of the invention for use in the preparation of a medicament or pharmaceutical composition for treatment of the disorders enumerated herein.

5 The invention also provides for the use of compound(s) of the invention for treating a disease. In particular, the invention provides for the use of compound(s) of the invention for treating the disorders enumerated herein.

10 The invention also provides for the use of compound(s) of the invention in the manufacture of a medicament or pharmaceutical composition. In particular, the invention provides for the use of compound(s) of the invention in the manufacture of a medicament or
15 pharmaceutical composition for the treatment of the disorders enumerated herein. Such pharmaceutical compositions include, as appropriate to the specific disorder, topical, systemic, oral or injectable formulations. It is further contemplated that the
20 compound(s) of the invention may be administered with an effective amount of a second therapeutic agent for the treatment of the enumerated disorders. A variety of pharmaceutical formulations and different delivery techniques are described in further detail below.

25 A preferred compound of the invention is a compound of formula (I):



where

- 30 n is 1-3;
 X is either O or S;
 R₁ is selected from the group consisting of C₁-C₆,

straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, aryl, heteroaryl, carbocycle, or heterocycle;

5 D is a bond, or a C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ alkenyl or C₂-C₁₀ alkynyl;

R₂ is a carboxylic acid or a carboxylic acid isostere; and

10 wherein said alkyl, alkenyl, alkynyl, aryl, heteroaryl, carbocycle, heterocycle, or carboxylic acid isostere is optionally substituted with one or more substituents selected from R³ and Z, where

15 R³ and Z are independently hydrogen, hydroxy, halo, haloalkyl, thiocarbonyl, alkoxy, alkenoxy, alkylaryloxy, aryloxy, arylalkyloxy, cyano, nitro, imino, alkylamino, aminoalkyl, sulfhydryl, thioalkyl, alkylthio, sulfonyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl or alkynyl, aryl, aralkyl, heteroaryl, carbocycle, heterocycle, and CO₂R⁷ where R⁷ is hydrogen or C₁-C₉ straight or branched chain alkyl or C₂-C₉ straight or branched chain alkenyl;

20 or a pharmaceutically acceptable salt, ester, or solvate thereof;

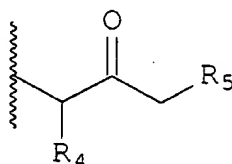
provided that:

25 when n=1, and D is a bond, and R₂ is COOH, then R₁ is not C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₅-C₇ cycloalkyl, C₅-C₇ cycloalkenyl, phenylamine, 2-(3,4-dichlorophenyl)ethyl, hydroxy, ethoxy, benzyl, or Ar₁, where Ar₁ is 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 1-pyridyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, or phenyl, and wherein said alkyl, alkenyl, cycloalkyl, cycloalkenyl, or Ar₁ are optionally substituted with one or more substituents selected from the group consisting of hydrogen, halo, hydroxyl, nitro,

35 trifluoromethyl, C₁-C₉ straight or branched alkyl, C₂-C₉

straight or branched alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, COOH, and amino; further provided that:

- when n=1, and D is a bond, and R₂ is the carboxylic acid isostere -CONZ(R³), and Z is hydrogen or C₁-C₆ alkyl, and R³ is phenyl, or C₂-C₆ straight or branched chain alkyl or alkenyl, wherein said alkyl is unsubstituted or substituted in one or more positions with Ar₂ as defined below, C₃-C₈ cycloalkyl, cycloalkyl connected by methyl or a C₂-C₆ straight or branched chain alkyl or alkenyl chain, C₁-C₄ alkyl ester, or Ar₃ where Ar₃ is selected from the group consisting of 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thiazolyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, or phenyl, having one to three substituents independently selected from the group consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched alkyl, C₂-C₆ straight or branched alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, and amino; wherein said alkyl ester is optionally substituted with phenyl; or R³ is the fragment:



- where R₄ is selected from the group consisting of straight or branched chain C₁-C₈ alkyl optionally substituted with C₃-C₈ cycloalkyl, benzyl, or Ar₂ as defined below, and where R₂ is COOZ or CONR⁶, where R⁶ is selected from the group consisting of hydrogen, C₁-C₆ straight or branched alkyl, and C₂-C₆ straight or branched alkenyl, and where R₅ is selected from the group consisting of phenyl, benzyl, C₁-C₆ straight or branched alkyl, and C₂-C₆ straight or branched alkenyl, where said alkyl or alkenyl is optionally substituted with phenyl;

then R_1 is not C_1-C_9 straight or branched chain alkyl, C_2-C_9 straight or branched chain alkenyl, substituted thiophene, or C_1-C_9 alkoxy, wherein said alkyl or alkenyl is optionally substituted in one or more positions with C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, or Ar_2 , where Ar_2 is defined below, where said alkyl, alkenyl, cycloalkyl or cycloalkenyl groups may be optionally substituted with C_1-C_4 alkyl, C_1-C_4 alkenyl, or hydroxy, and where Ar_2 is 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, or phenyl, having one to three substituents selected from the group consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C_1-C_6 straight or branched alkyl, C_2-C_6 straight or branched alkenyl, C_1-C_4 alkoxy, C_2-C_4 alkenyloxy, phenoxy, benzyloxy, and amino;

further provided that:

when $n=1$, and X is O, and D is a bond, and R_2 is $-CONH_2$, then R_1 is not methyl, ethyl, iso-propyl, iso-butyl, iso-pentyl, 4-methylpentyl, indolyl, phenyl, or hydroxyphenyl;

further provided that:

when $n=1$, and X is O, and D is a bond, and R_2 is cyano, then R_1 is not methyl;

further provided that:

when $n=2$, and X is O, and D is a bond, and R_2 is $CONZ(R^3)$, and R_1 is ethoxy, then R^3 or Z is not halo-substituted phenyl;

further provided that:

when $n=2$, and X is O, and D is a bond, and R_2 is $CONZ(R^3)$ and R_1 is substituted thiophene or tetrahydropyranoxy, or methoxy, then R^3 or Z is not C_1-C_4 alkyl ester substituted ethyl;

further provided that:

when $n=2$, and X is O, and D is a bond, and R_2 is $CONZ(R^3)$ and R_1 is ethoxy, then R^3 or Z is not 4-chlorophenyl;

further provided that:

when $n=2$, and X is O, and D is a bond, and R_2 is $\text{CONZ}(R^3)$ and R_1 is cyclohexyl, then R^3 or Z is not ethyl or propyl substituted with phenyl;

further provided that:

- 5 when D is -CH_2 , then R_2 is not -OMe , -NHMe , or substituted -NHcyclohexyl ;

further provided that:

when D is CH_2 , and R_2 is -OH ,

then R_1 is not phenyl or pyrrolidinemethanol;

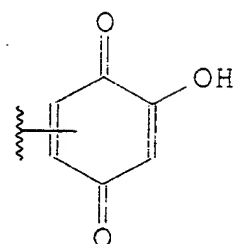
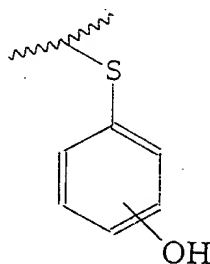
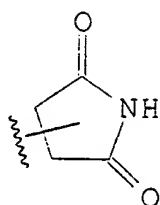
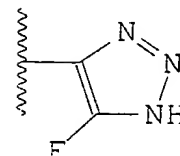
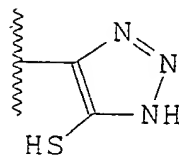
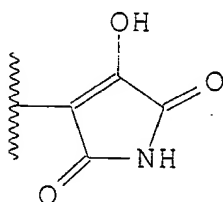
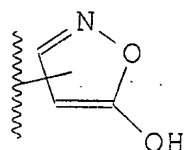
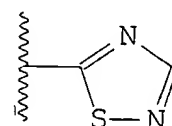
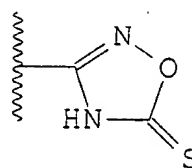
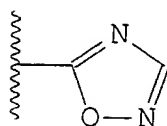
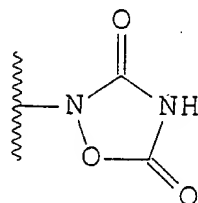
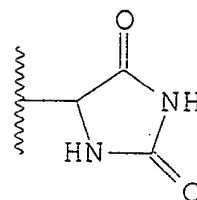
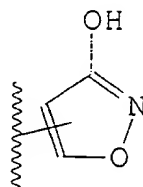
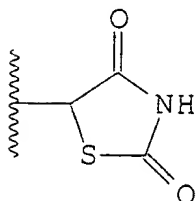
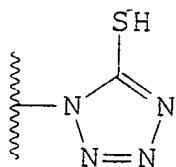
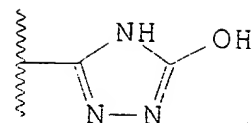
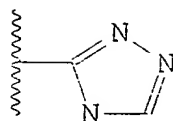
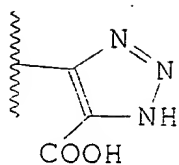
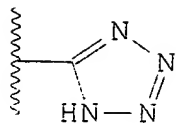
- 10 further provided that:

when $n=2$, and X is O, and D is a bond, and R_2 is COOH ,

- then R_1 is not methyl, tert-butyl, 1,1-dimethyl-2-methyl-propyl, 1,1-dimethyl-propyl, methoxy, ethoxy, phenyl, tetrahydropyranoxy substituted $\text{C}_4\text{-C}_6$ alkyl, 1-methyl-1-methoxyamide, 1-methylcyclohexyl, 3-iodophenyl, 3-methyl ester-cyclopentyl, 1,1-dimethyl-6-phenyl-hex-3,5-dioxy, or trimethoxyphenyl.
- 15

- Preferred embodiments of this invention are where R_2 is a carbocycle or heterocycle containing any combination of CH_2 , O, S, or N in any chemically stable oxidation state, where any of the atoms of said ring structure are optionally substituted in one or more positions with R^3 .
- 20

Especially preferred embodiments of this invention are where R_2 is selected from the group below:



where the atoms of said ring structure may be optionally substituted at one or more positions with R³.

Another preferred embodiment of this invention is where R₂ is selected from the group consisting of -COOH, -SO₃H, -SO₂HNR³, -PO₂(R³)₂, -CN, -PO₃(R³)₂, -OR³, -SR³, -NHCOR³, -N(R³)₂, -CON(R³)₂, -CONH(O)R³, -CONHNHSO₂R³, -COHNSO₂R³, and -CONR³CN.

Preferred embodiments of this invention are:

(2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-hydroxymethyl pyrrolidine;

(2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine tetrazole;

(2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidine carbonitrile;

(2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-aminocarbonyl piperidine;

[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-N-(2-thienylcarbonylamino)-formamide;

3,3-Dimethyl-1-{2-[(4-nitrophenoxy)methyl]pyrrolidinyl} pentane-1,2-dione;

2-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]ethane nitrile;

1-[2-(3-Ethyl(1,2,4-oxadiazol-5-yl))pyrrolidinyl]-3,3-dimethylpentane-1,2-dione;

1-{2-[3-(4-Fluorophenyl)(1,2,4-oxadiazol-5-yl)]pyrrolidinyl}-3,3-dimethylpentane-1,2-dione;

3,3-Dimethyl-1-[2-(3-methyl(1,2,4-oxadiazol-5-yl))pyrrolidinyl]pentane-1,2-dione;

[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-N-[(methylsulfonyl)amino]-formamide;

[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-N-[(4-methylphenyl)sulfonyl]-amino)formamide;

[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-N-[(4-fluorophenyl)sulfonyl]-amino)formamide;

1-[Benzylsulfonyl]-2-(pyrrolidinylmethyl)pyrrolidine;

(2S)-3,3-Dimethyl-1-[2-(5-sulfanyl(4H-1,2,4-triazol-3-yl))
pyrrolidinyl]-pentane-1,2-dione;

(2S)-3,3-Dimethyl-1-[2-(pyrrolidinylmethyl)pyrrolidinyl]
pentane-1,2-dione;

5 (2S)-N-[(Aminothioxomethyl)amino][1-(3,3-dimethyl-2-oxo
pentanoyl)pyrrolidin-2-yl]formamide;

(2S)-1-[2-(Benzotriazol-1-ylcarbonyl)pyrrolidinyl]-3,3-
dimethylpentane-1,2-dione;

10 N-Amino-2-[2-(N-aminocarbamoyl)pyrrolidinyl]-2-oxo
ethanamide;

2-[1-(3,3-Dimethyl-2-oxopentanoyl)-2-piperidyl]acetic
acid;

1-(2-([4-(2H-Benzo[3,4-d]1,3-dioxolen-5-ylmethyl)
piperazinyl]carbonyl)pyrrolidinyl)-3,3-dimethylpentane-
15 1,2-dione; and,

1-[2-([4-[Bis(4-fluorophenyl)methyl]piperazinyl]carbonyl)
pyrrolidinyl]-3,3-dimethylpentane-1,2-dione.

BRIEF DESCRIPTION OF THE DRAWINGS

20 Figure 1 is a photograph of C57 Black 6 mice before
being shaved for the hair regeneration experiment.

Figure 2 is a photograph of mice treated with a
vehicle after six weeks. Figure 2 shows that less than 3%
of the shaved area is covered with new hair growth when
25 the vehicle (control) is administered.

Figure 3 is a bar graph illustrating relative hair
growth on shaved mice treated with N-heterocyclic
carboxylic acids or carboxylic acid isosteres at 1μmole
per milliliter three times per week. Hair growth was
30 evaluated after 14 days of treatment.

Figure 4 A, B, and C show that GPI 1046 protects
ganglion cells against degeneration following retinal
ischemia.

Figure 5 shows that GPI 1046 prevents degeneration of
35 optic nerve axons and myelin following retinal ischemia.

Figure 6 shows that GPI 1046 provides moderate protection against retinal ganglion cell death after optic nerve transection.

Figure 7 shows that GPI 1046 treatment duration significantly affects the process of optic nerve axonal degeneration after transection.

Figure 8 shows that GPI 1046 treatment produces a greater effect on optic nerve axons than ganglion cell bodies.

Figure 9 shows that GPI 1046 treatment for 28 days after optic nerve transection prevents myelin degeneration in the proximal stump.

Figure 10 shows that FKBP-12 immunohistochemistry labels oligodendroglia (large dark cells with fibrous processes), the cells which produce myelin, located between the fascicles of optic nerve fibers, and also some optic nerve axons.

Figure 11 shows GPI 1046 treatment for 28 days after optic nerve transection prevents myelin degeneration in the distal stump.

Figure 12 shows that 28 day treatment with GPI 1046 treatment beginning 8 weeks after onset of streptozotocin induced diabetes decreases the extent of neovascularization in the inner and outer retina and protects neurons in the inner nuclear layer (INL) and ganglion cell layer (GCL) from degeneration.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Alkyl" means a branched or unbranched saturated hydrocarbon chain comprising a designated number of carbon atoms. For example, C₁-C₆ straight or branched alkyl hydrocarbon chain contains 1 to 6 carbon atoms, and includes but is not limited to substituents such as methyl, ethyl, propyl, iso-propyl, butyl, iso-butyl, tert-

butyl, n-pentyl, n-hexyl, and the like. It is also contemplated as within the scope of the present invention that "alkyl" may also refer to a hydrocarbon chain wherein any of the carbon atoms of said alkyl are optionally replaced with O, NH, S, or SO₂. For example, carbon 2 of n-pentyl can be replaced with O to form propyloxymethyl.

"Alkenyl" means a branched or unbranched unsaturated hydrocarbon chain comprising a designated number of carbon atoms. For example, C₂-C₆ straight or branched alkenyl hydrocarbon chain contains 2 to 6 carbon atoms having at least one double bond, and includes but is not limited to substituents such as ethenyl, propenyl, iso-propenyl, butenyl, iso-butenyl, tert-butenyl, n-pentenyl, n-hexenyl, and the like. It is also contemplated as within the scope of the present invention that "alkenyl" may also refer to an unsaturated hydrocarbon chain wherein any of the carbon atoms of said alkenyl are optionally replaced with O, NH, S, or SO₂. For example, carbon 2 of 4-pentene can be replaced with O to form (2-propene)oxymethyl.

"Alkoxy" means the group -OR wherein R is alkyl as herein defined. Preferably, R is a branched or unbranched saturated hydrocarbon chain containing 1 to 6 carbon atoms.

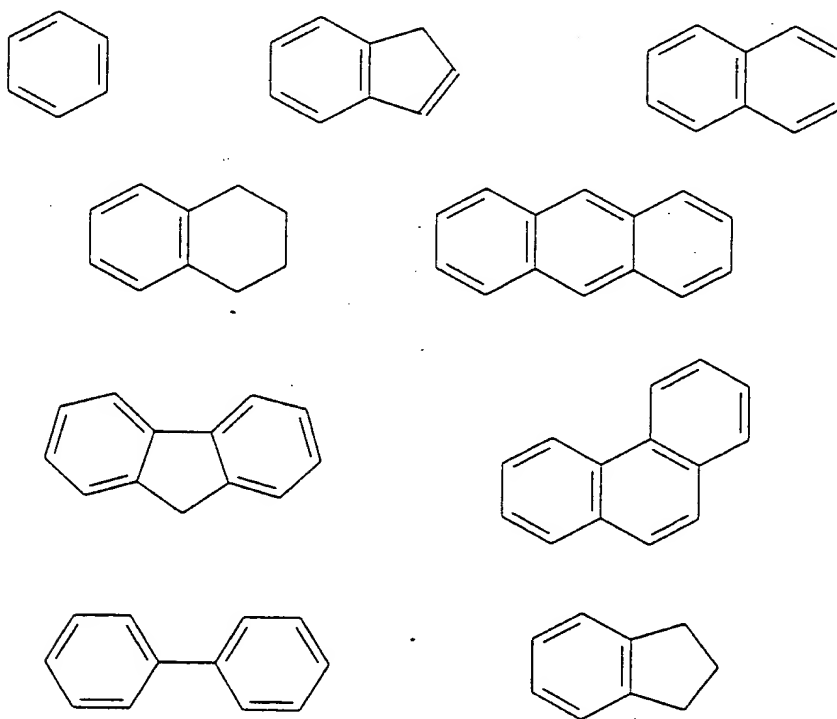
Specifically, the term "carbocycle" or refers to an organic cyclic moiety in which the cyclic skeleton is comprised of only carbon atoms whereas the term "heterocycle" refers to an organic cyclic moiety in which the cyclic skeleton contains one or more heteroatoms selected from nitrogen, oxygen, or sulfur and which may or may not include carbon atoms.

Thus, the term "carbocycle" refers to a carbocyclic moiety containing the indicated number of carbon atoms. The term "C₃-C₈ cycloalkyl", therefore, refers to an organic cyclic substituent in which three to eight carbon atoms form a three, four, five, six, seven, or eight-

membered ring, including, for example, a cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl ring. As used herein, "carbocycle" may also refer to two or more cyclic ring systems which are fused to form, for example bicyclic, tricyclic, or other similar bridged substituents (e.g. adamantyl).

"Aryl" refers to an aromatic carbocyclic group having a single ring, for example a phenyl ring; multiple rings, for example biphenyl; or multiple condensed rings in which at least one ring is aromatic, for example naphthyl, 1,2,3,4-tetrahydronaphthyl, anthryl, or phenanthryl, which can be unsubstituted or substituted with one or more other substituents as defined above. The substituents attached to a phenyl ring portion of an aryl moiety in the compounds of Formula (I) may be configured in the ortho-, meta-, or para- orientations.

Examples of typical aryl moieties included in the scope of the present invention may include, but are not limited to, the following:



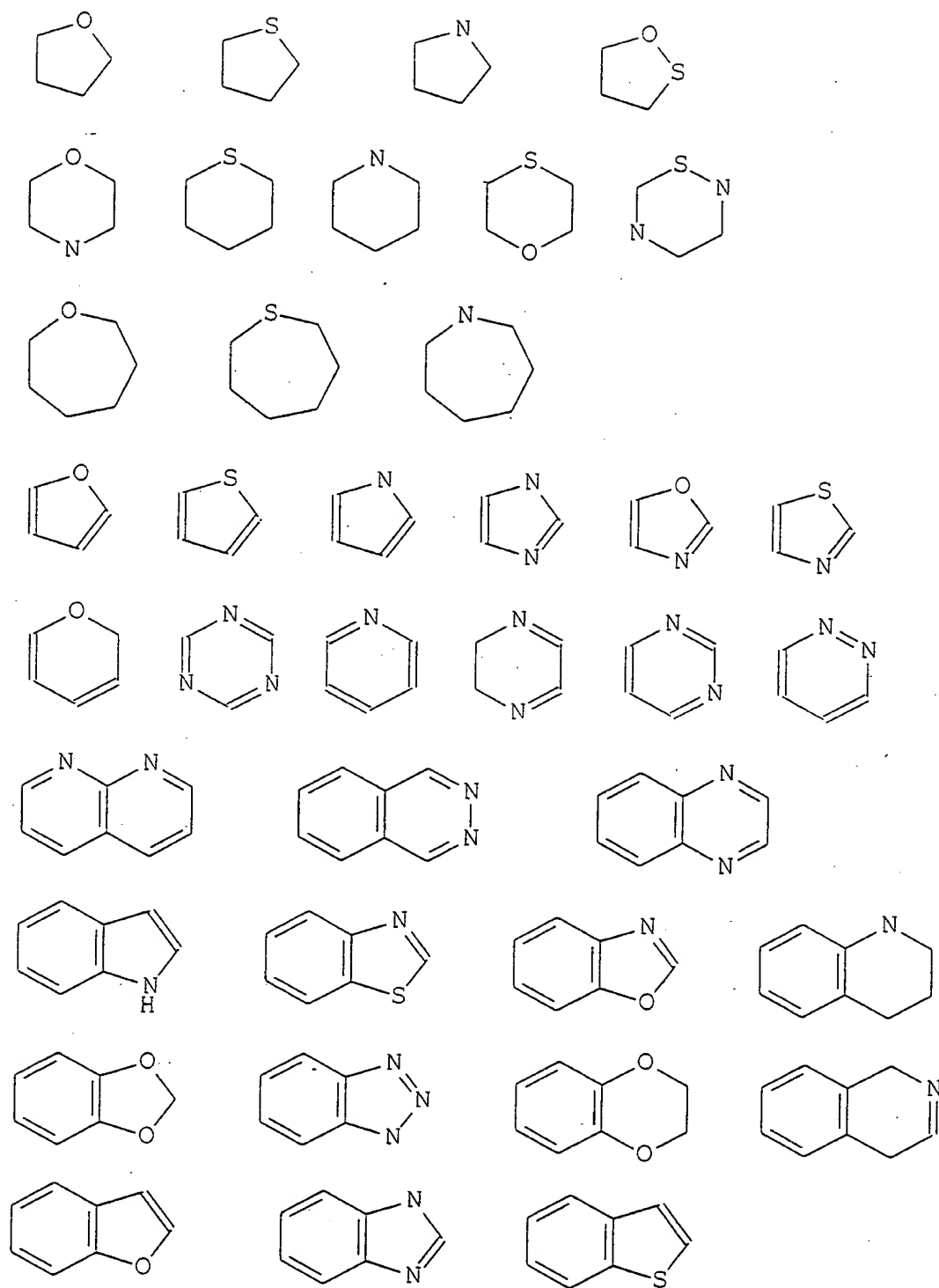
"Aralkyl" refers to alkyl or alkylene (alkenyl) chain which is substituted with aryl, heteroaryl, carbocycle or heterocycle, or alternatively one or more aryl, heteroaryl, carbocycle, or heterocycle(s) which is/are substituted with alkyl or alkenyl, i.e. 'Alkyl/alkylene which is substituted with Ar' or 'Ar which is substituted with alkyl/alkylene'.

"Heterocycle" refers to a saturated, unsaturated, or aromatic carbocyclic group having a single ring, multiple rings, or multiple condensed rings, and having at least one hetero atom such as nitrogen, oxygen, or sulfur within at least one of the rings. "Heteroaryl" refers to a heterocycle in which at least one ring is aromatic. Any of the heterocyclic or heteroaryl groups can be unsubstituted or optionally substituted with one or more groups as defined above. Further, bi- or tri-cyclic heteroaryl moieties may comprise at least one ring which is either completely or partially saturated.

As one skilled in the art will appreciate, such heterocyclic moieties may exist in several isomeric forms, all of which are encompassed by the present invention. For example, a 1,3,5-triazine moiety is isomeric to a 1,2,4-triazine group. Such positional isomers are to be considered within the scope of the present invention. Likewise, the heterocyclic or heteroaryl groups can be bonded to other moieties in the compounds of the present invention. The point(s) of attachment to these other moieties is not to be construed as limiting on the scope of the invention. Thus, by way of example, a pyridyl moiety may be bound to other groups through the 2-, 3-, or 4-position of the pyridyl group. All such configurations are to be construed as within the scope of the present invention.

Examples of heterocyclic or heteroaryl moieties included in the scope of the present invention may

include, but are not limited to, the following:



"Halo" means at least one fluoro, chloro, bromo, or iodo moiety.

The term "pharmaceutically acceptable salt, ester, or solvate" refers to salt, ester, or solvates of the subject compounds which possess the desired pharmacological activity and which are neither biologically nor otherwise undesirable. The salt, ester, or solvates can be formed with inorganic or organic acids such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, gluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, naphthylate, 2-naphthalenesulfonate, nicotinate, oxalate, sulfate, thiocyanate, tosylate and undecanoate. Base salt, ester, or solvates include ammonium salts, alkali metal salts such as lithium, sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups can be quarternized with such agents as: 1) lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; 2) dialkyl sulfates like dimethyl, diethyl, dibutyl and diamyl sulfates; 3) long chain alkyls such as decyl, lauryl, myristyl and stearyl substituted with one or more halide such as chloride, bromide and iodide; and 4) aralkyl halides like benzyl and phenethyl bromide and others.

The compounds of this invention may possess at least one asymmetric center and thus can be produced as mixtures of stereoisomers or as individual enantiomers or

diastereomers. The individual stereoisomers may be obtained by using an optically active starting material, by resolving a racemic or non-racemic mixture of an intermediate at some appropriate stage of the synthesis, or by resolution of the compound of formula (I). It is understood that the individual stereoisomers as well as mixtures (racemic and non-racemic) of stereoisomers are encompassed by the scope of the present invention. The S-stereoisomer at atom 1 of formula I is a most preferred embodiment of the invention.

"Stereoisomers" are isomers that differ only in the way the atoms are arranged in space.

"Isomers" are different compounds that have the same molecular formula and includes cyclic isomers such as (iso)indole and other isomeric forms of cyclic moieties.

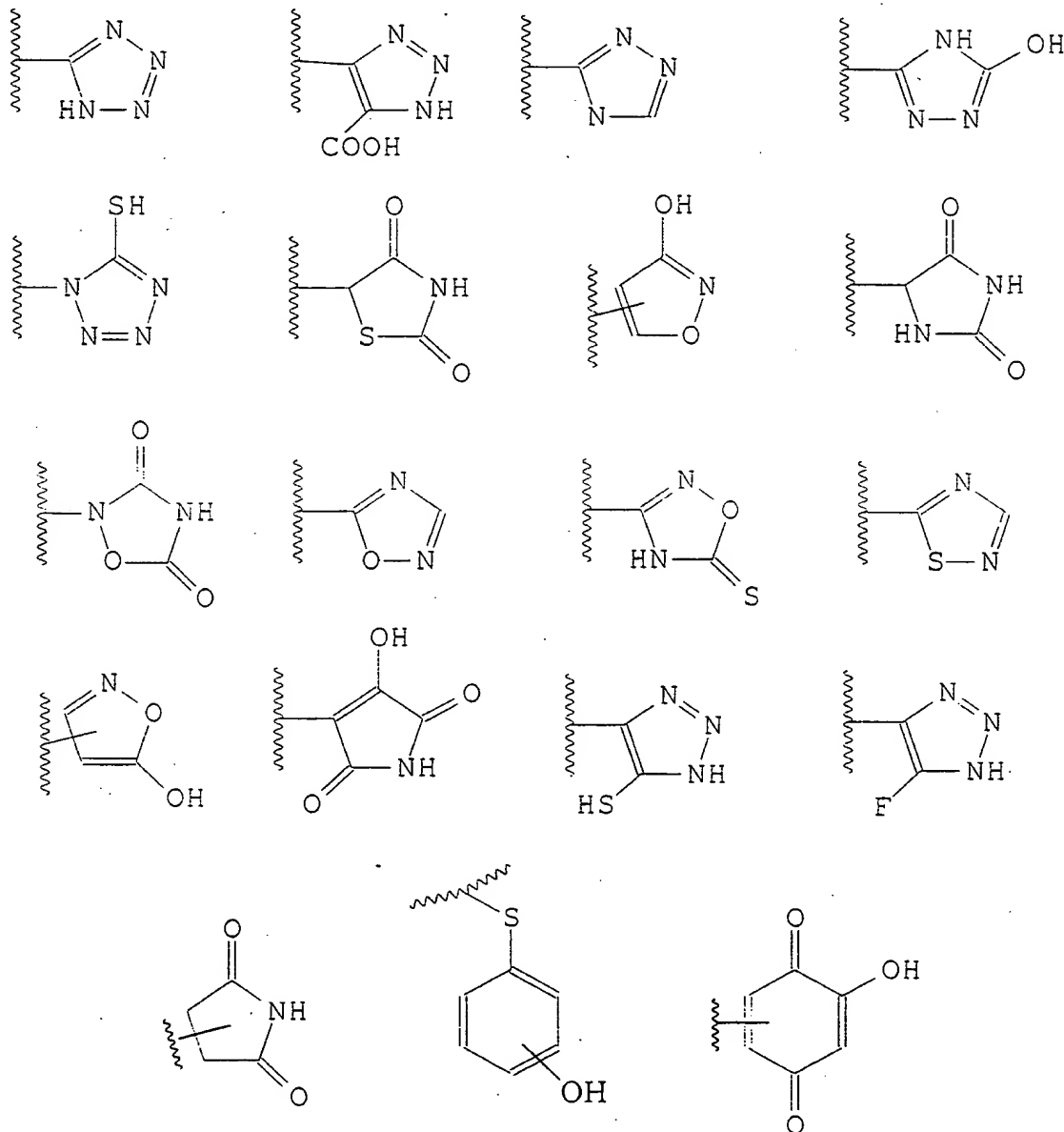
"Enantiomers" are a pair of stereoisomers that are non-superimposable mirror images of each other.

"Diastereoisomers" are stereoisomers which are not mirror images of each other.

"Racemic mixture" means a mixture containing equal parts of individual enantiomers. "Non-racemic mixture" is a mixture containing unequal parts of individual enantiomers or stereoisomers.

"Isosteres" are different compounds that have different molecular formulae but exhibit the same or similar properties. For example, tetrazole is an isostere of carboxylic acid because it mimics the properties of carboxylic acid even though they both have very different molecular formulae. Tetrazole is one of many possible isosteric replacements for carboxylic acid. Other carboxylic acid isosteres contemplated by the present invention include $-\text{COOH}$, $-\text{SO}_3\text{H}$, $-\text{SO}_2\text{HNR}^3$, $-\text{PO}_2(\text{R}^3)_2$, $-\text{CN}$, $-\text{PO}_3(\text{R}^3)_2$, $-\text{OR}^3$, $-\text{SR}^3$, $-\text{NHCOR}^3$, $-\text{N}(\text{R}^3)_2$, $-\text{CON}(\text{R}^3)_2$, $-\text{CONH}(\text{O})\text{R}^3$, $-\text{CONHNHSO}_2\text{R}^3$, $-\text{COHNSO}_2\text{R}^3$, and $-\text{CONR}^3\text{CN}$. In addition, carboxylic acid isosteres can include 5-7 membered

- carbocycles or heterocycles containing any combination of CH₂, O, S, or N in any chemically stable oxidation state, where any of the atoms of said ring structure are optionally substituted in one or more positions. The
- 5 following structures are non-limiting examples of preferred carbocyclic and heterocyclic isosteres contemplated by this invention.



where the atoms of said ring structure may be optionally substituted at one or more positions with R^3 . The present invention contemplates that when chemical substituents are added to a carboxylic isostere then the inventive compound

5 retains the properties of a carboxylic isostere.

The present invention contemplates that when a carboxylic isostere is optionally substituted with one or more moieties selected from R^3 , then the substitution cannot eliminate the carboxylic acid isosteric properties of the

10 inventive compound. The present invention contemplates that the placement of one or more R^3 substituents upon a carbocyclic or heterocyclic carboxylic acid isostere shall not be permitted at one or more atom(s) which maintain(s) or is/are integral to the carboxylic acid isosteric

15 properties of the inventive compound, if such substituent(s) would destroy the carboxylic acid isosteric properties of the inventive compound.

Other carboxylic acid isosteres not specifically exemplified or described in this specification are also

20 contemplated by the present invention.

It is understood that where chemical substitution is indicated then the chemical substituent chosen would form a sufficiently stable compound.

The term "preventing neurodegeneration" as used

25 herein includes the ability to inhibit or prevent neurodegeneration in patients newly diagnosed as having a neurodegenerative disease, or at risk of developing a new degenerative disease and for inhibiting or preventing further neurodegeneration in patients who are already

30 suffering from or have symptoms of a neurodegenerative disease when the compounds are given concurrently.

The term "treatment" as used herein covers any treatment of a disease and/or condition in an animal, particularly a human, and includes:

35 (i) preventing a disease and/or condition from

occurring in a subject which may be predisposed to the disease and/or condition but has not yet been diagnosed as having it;

(ii) inhibiting the disease and/or condition, i.e.,
5 arresting its development; or

(iii) relieving the disease and/or condition, i.e., causing regression of the disease and/or condition.

The system used in naming the compounds of the present invention is shown below, using a compound of
10 formula I as an example.

A compound of the present invention, especially formula I, wherein n is 1, X is O, D is a bond, R₁ is 1,1-dimethylpropyl, and R₂ is -CN, is named (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarbonitrile.

15 "Alopecia" refers to deficient hair growth and partial or complete loss of hair, including without limitation androgenic alopecia (male pattern baldness), toxic alopecia, alopecia senilis, alopecia areata, alopecia pelada and trichotillomania. Alopecia results
20 when the pilar cycle is disturbed. The most frequent phenomenon is a shortening of the hair growth or anagen phase due to cessation of cell proliferation. This results in an early onset of the catagen phase, and consequently a large number of hairs in the telogen phase
25 during which the follicles are detached from the dermal papillae, and the hairs fall out. Alopecia has a number of etiologies, including genetic factors, aging, local and systemic diseases, febrile conditions, mental stresses, hormonal problems, and secondary effects of drugs.

30 "Pilar cycle" refers to the life cycle of hair follicles, and includes three phases:

- (1) the anagen phase, the period of active hair growth which, insofar as scalp hair is concerned, lasts about three to five years;
- 35 (2) the catagen phase, the period when growth stops

and the follicle atrophies which, insofar as scalp hair is concerned, lasts about one to two weeks; and

- 5 (3) the telogen phase, the rest period when hair progressively separates and finally falls out which, insofar as scalp hair is concerned, lasts about three to four months.

10 Normally 80 to 90 percent of the follicles are in the anagen phase, less than 1 percent being in the catagen phase, and the rest being in the telogen phase. In the telogen phase, hair is uniform in diameter with a slightly bulbous, non-pigmented root. By contrast, in the anagen phase, hair has a large colored bulb at its root.

15 "Promoting hair growth" refers to maintaining, inducing, stimulating, accelerating, or revitalizing the germination of hair.

"Treating alopecia" refers to:

- 20 (i) preventing alopecia in an animal which may be predisposed to alopecia; and/or
(ii) inhibiting, retarding or reducing alopecia; and/or
(iii) promoting hair growth; and/or
(iv) prolonging the anagen phase of the hair cycle; and/or
25 (v) converting vellus hair to growth as terminal hair. Terminal hair is coarse, pigmented, long hair in which the bulb of the hair follicle is seated deep in the dermis. Vellus hair, on the other hand, is fine, thin, non-pigmented short hair in which the hair bulb is located
30 superficially in the dermis. As alopecia progresses, the hairs change from the terminal to the vellus type.

"Eye" refers to the anatomical structure responsible for vision in humans and other animals, and encompasses the following anatomical structures, without limitation:
35 lens, vitreous body, ciliary body, posterior chamber,

anterior chamber, pupil, cornea, iris, canal of Schlemm, zonules of Zinn, limbus, conjunctiva, choroid, retina, central vessels of the retina, optic nerve, fovea centralis, macula lutea, and sclera.

5 "Neopsic factors" or "neopsics" refers to compounds useful in treating vision loss, preventing vision degeneration, or promoting vision regeneration.

"Neopsis" refers to the process of treating vision loss, preventing vision degeneration, or promoting vision
10 regeneration.

-- "Ophthalmological" refers to anything about or concerning the eye, without limitation, and is used interchangeably with "ocular," "ophthalmic,"
15 "ophthalmologic," and other such terms, without limitation.

"Preventing vision degeneration" as used herein includes the ability to prevent degeneration of vision in patients newly diagnosed as having a degenerative disease affecting vision, or at risk of developing a new
20 degenerative disease affecting vision, and for preventing further degeneration of vision in patients who are already suffering from or have symptoms of a degenerative disease affecting vision.

"Promoting vision regeneration" refers to
25 maintaining, improving, stimulating or accelerating recovery of, or revitalizing one or more components of the visual system in a manner which improves or enhances vision, either in the presence or absence of any ophthalmologic disorder, disease, or injury.

30 "Vision", as used herein, refers to the ability of humans and other animals to process images, and is used interchangeably with "sight", "seeing", and other such terms, without limitation.

"Vision disorder" refers to any disorder that affects
35 or involves vision, including without limitation visual

impairment, orbital disorders, disorders of the lacrimal apparatus, disorders of the eyelids, disorders of the conjunctiva, disorders of the cornea, cataracts, disorders of the uveal tract, disorders of the optic nerve or visual pathways, free radical induced eye disorders and diseases, immunologically-mediated eye disorders and diseases, eye injuries, and symptoms and complications of eye disease, eye disorder, or eye injury.

"Visual impairment" refers to any dysfunction in vision including without limitation disturbances or diminution in vision (e.g., binocular, central, peripheral, scotopic), visual acuity for objects near and for, visual field, ocular motility, color perception, adaptation to light and dark, accommodation, refraction, and lacrimation. See *Physicians' Desk Reference (PDR) for Ophthalmology*, 16th Edition, 6:47 (1988).

"Enhancing memory performance" refers to improving or increasing the mental faculty by which to register, retain or recall past experiences, knowledge, ideas, sensations, thoughts or impressions.

"Memory impairment" refers to a diminished mental registration, retention or recall of past experiences, knowledge, ideas, sensations, thoughts or impressions. Memory impairment may affect short and long-term information retention, facility with spatial relationships, memory (rehearsal) strategies, and verbal retrieval and production. Common causes of memory impairment are age, severe head trauma, brain anoxia or ischemia, alcoholic-nutritional diseases, and drug intoxications. Examples of memory impairment include, without limitation, benign forgetfulness, amnesia and any disorder in which memory deficiency is present, such as Korsakoff's amnesic psychosis, dementia and learning disorders.

The term "middle-ear" refers to the space between the

tympenic membrane and the inner ear. This location is external to all inner ear tissue and an invasive procedure might not be required to access this region if a formulation capable of penetrating through the tympanic membrane is administered. Otherwise, the material will be introduced to the middle ear by injection through the tympanic membrane or, in case repeated administrations are needed, a hole can be made in the tympanic membrane. An opening in the tympanic membrane is a frequent procedure, performed on an office-visit basis, in cases such as infections of the middle ear (usually in children). The opening generally closes spontaneously after a few days.

The term "neurotrophic" as used herein includes without limitation the ability to stimulate neuronal regeneration or growth and/or the ability to prevent or treat neurodegeneration.

The term "non-immunosuppressive" refers to the inability of the compounds of the present invention to trigger an immune response when compared to a control such as FK506 or cyclosporin A. Assays for determining immunosuppression are well known to those of ordinary skill in the art. Specific non-limiting examples of well known assays include PMA and OKT3 assays wherein mitogens are used to stimulate proliferation of human peripheral blood lymphocytes (PBC). Compounds added to such assay systems are evaluated for their ability to inhibit such proliferation.

The term "small molecule" refers to the molecular weight of the compounds of the invention as compared to FK506. Thus, the term "small molecule" includes molecular weights less than about 800 Daltons (m.w.), and novel subranges or limits below the same including about 100 to about 750 m.w., about 150 to about 500 m.w., about 150 to about 350 m.w., about 200 to about 300 m.w., about 210 to about 280 m.w., about 220 to about 260, and about 240

m.w.. The term "spatially small molecule" refers to the capability of the compounds to fit entirely or substantially within the binding cavity of FKBP-12 as compared to FK506.

5

Utility of the Compounds of the Invention

The present invention relates to the surprising discovery that carboxylic acid or carboxylic acid isostere compounds are neurotrophic, are able to treat alopecia, are able to treat vision and memory disorders, and are able to treat sensorineural hearing loss. Accordingly, a novel class of compounds are provided. A preferred feature of the compounds of the present invention is that they do not exert any significant immunosuppressive activity.

Preferred compounds of the present invention contain carboxylic acid moieties and other isosteric replacements for carboxylic acid moieties, of which several examples are specified herein. Other isosteric replacements for carboxylic acid moieties, known to those skilled in the art of medicinal chemistry, are within the scope of the invention if not otherwise specified.

The compounds of this invention can be periodically administered to a patient undergoing treatment for neurological disorders or for other reasons in which it is desirable to stimulate neuronal regeneration and growth, such as in various peripheral neuropathic and neurological disorders relating to neurodegeneration. The compounds of this invention can also be administered to mammals other than humans for treatment of various mammalian neurological disorders.

The novel compounds of the present invention possess an excellent degree of neurotrophic activity. This activity is useful in the stimulation of damaged neurons, the promotion of neuronal regeneration, the prevention of

neurodegeneration, and in the treatment of several neurological disorders known to be associated with neuronal degeneration and peripheral neuropathies. The neurological disorders that may be treated include but are not limited to: trigeminal neuralgia, glossopharyngeal neuralgia, Bell's Palsy, myasthenia gravis, muscular dystrophy, amyotrophic lateral sclerosis, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed invertebrate disk syndromes, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathic such as those caused by lead, dapsone, ticks, prophyria, or Gullain-Barré syndrome, multiple sclerosis, stroke and ischemia associate with stroke, neural paropathy, other neurodegenerative diseases, motor neuron diseases, sciatic crush, peripheral neuropathy, particularly neuropathy associate with diabetes, spinal cord injuries and facial nerve crush, Huntington's Disease, Alzheimer's disease, and Parkinson's disease.

The above discussion relating to the utility and administration of the compounds of the present invention also applies to the pharmaceutical compositions of the present invention.

The term "pharmaceutically acceptable carrier" as used herein refers to any carrier, diluent, excipient, suspending agent, lubricating agent, adjuvant, vehicle, delivery system, emulsifier, disintegrant, absorbant, preservative, surfactant, colorant, flavorant, or sweetener.

For these purposes the compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and

vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneally, intrathecally, intraventricularly, intrasternal and intracranial injection or infusion techniques.

For oral administration; the compounds of the present invention may be provided in any suitable dosage form known in the art. For example, the compositions may be incorporated into tablets, powders, granules, beads, chewable lozenges, capsules, liquids, aqueous suspensions or solutions, or similar dosage forms, using conventional equipment and techniques known in the art. Tablet dosage forms are preferred. Tablets may contain carriers such as lactose and corn starch, and/or lubricating agents such as magnesium stearate. Capsules may contain diluents including lactose and dried corn starch. Aqueous suspensions may contain emulsifying and suspending agents combined with the active ingredient.

When preparing dosage form incorporating the compositions of the invention, the compounds may also be blended with conventional excipients such as binders, including gelatin, pregelatinized starch, and the like; lubricants, such as hydrogenated vegetable oil, stearic acid, and the like; diluents, such as lactose, mannose, and sucrose; disintegrants, such as carboxymethylcellulose and sodium starch glycolate; suspending agents, such as povidone, polyvinyl alcohol, and the like; absorbents, such as silicon dioxide; preservatives, such as methylparaben, propylparaben, and sodium benzoate; surfactants, such as sodium lauryl sulfate, polysorbate 80, and the like; colorants such as F.D. & C. dyes and lakes; flavorants; and sweeteners.

Compositions and methods of the invention also may utilize controlled release technology. Thus, for example, the inventive compounds may be incorporated into a

hydrophobic polymer matrix for controlled release over a period of days. Such controlled release films are well known to the art. Particularly preferred are transdermal delivery systems. Other examples of polymers commonly employed for this purpose that may be used in the present invention include nondegradable ethylene-vinyl acetate copolymer and degradable lactic acid-glycolic acid copolymers which may be used externally or internally. Certain hydrogels such as poly(hydroxyethylmethacrylate) or poly(vinylalcohol) also may be useful, but for shorter release cycles than the other polymer releases systems, such as those mentioned above.

To be effective therapeutically as central nervous system targets, the compounds of the present invention should readily penetrate the blood-brain barrier when peripherally administered. Compounds which cannot penetrate the blood-brain barrier can be effectively administered by an intraventricular route or other appropriate delivery system suitable for administration to the brain.

The compounds of the present invention may be administered in the form of sterile injectable preparations, for example, as sterile injectable aqueous or oleaginous suspensions. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparations may also be sterile injectable solutions or suspensions in non-toxic parenterally-acceptable diluents or solvents, for example, as solutions in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as solvents or suspending mediums. For this purpose, any bland fixed oil may be employed including

synthetic mono- or di-glycerides. Fatty acids such as oleic acid and its glyceride derivatives, including olive oil and castor oil, especially in their polyoxyethylated versions, are useful in the preparation of injectables.

5 These oil solutions or suspensions may also contain long-chain alcohol diluents or dispersants.

The compounds of this invention may also be administered rectally in the form of suppositories. These compositions can be prepared by mixing the drug with a
10 suitable non-irritating excipient which is solid at room temperature, but liquid at rectal temperature and, therefore, will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

15 The compounds of this invention may also be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin, or the lower intestinal
20 tract. Suitable topical formulations are readily prepared for each of these areas.

For topical application to the eye, or ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or,
25 preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses the compounds may be formulated in an ointment such as petrolatum.

30 For topical application to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol,
35 polyoxyethylene polyoxypropylene compound, emulsifying wax

and water. Alternatively, the compounds can be formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of about 0.1 mg to about 10,000 mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels of about 0.1 mg to about 1,000 mg. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Typically, *in vitro* dosage-effect results provide useful guidance on the proper doses for patient administration. Studies in animal models are also helpful. The considerations for determining the proper dose levels are well known in the art.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated and form of administration.

To effectively treat alopecia or promote hair growth, the compounds used in the inventive methods and pharmaceutical compositions must readily affect the targeted areas. For these purposes, the compounds are preferably administered topically to the skin.

For topical application to the skin, the compounds can be formulated into suitable ointments containing the

compounds suspended or dissolved in, for example, mixtures with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated into suitable lotions or creams containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl ester wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The compounds can be administered with other hair revitalizing agents. Specific dose levels for the other hair revitalizing agents will depend upon the factors previously stated and the effectiveness of the drug combination. Other routes of administration known in the pharmaceutical art are also contemplated by this invention.

Pharmaceutical Compositions of the Present Invention

The present invention relates to a pharmaceutical composition comprising:

- (i) an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere compound; and
- (ii) a pharmaceutically acceptable carrier.

The present invention also relates to a pharmaceutical composition comprising:

- (i) an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere compound for treating neurodegenerative diseases, neurological disorders, and nerve damage, or promoting nerve growth in an animal; and
- (ii) a pharmaceutically acceptable carrier.

The present invention also relates to a pharmaceutical composition comprising:

- 5 (i) an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere compound for treating alopecia or promoting hair growth in an animal; and
- (ii) a pharmaceutically acceptable carrier.

The present invention also relates to a pharmaceutical composition comprising:

- 10 (i) an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere compound for treating a vision disorder, improving vision, treating memory impairment, or enhancing memory performance in an animal; and
- 15 (ii) a pharmaceutically acceptable carrier.

The present invention also relates to a pharmaceutical composition comprising:

- 20 (i) an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere compound for treating sensorineural hearing loss in an animal; and
- (ii) a pharmaceutically acceptable carrier.

For pharmaceutical compositions directed specifically to neurotrophic medical indications, one or more
25 additional neurotrophic factor(s) or neurotrophic agent(s) may be administered in combination with, or otherwise included in, the composition. The compounds can be administered with other neurotrophic agents such as
30 neurotrophic growth factor, brain derived growth factor, glial derived growth factor, ciliary neurotrophic factor, insulin growth factor and active truncated derivatives thereof, acidic fibroblast growth factor, basic fibroblast growth factor, platelet-derived growth factors,
neurotrophin-3 and neurotrophin 4/5. The dosage level of
35 other neurotrophic drugs will depend upon the factors

previously stated and the neurotrophic effectiveness of the drug combination.

Similarly pharmaceutical compositions directed specifically to hair loss related medical indications may also be administered in combination with an additional agent(s).

Methods of the Present Invention

The present invention relates to the use of any of the compounds seen in Tables I, II, III, IV, other compounds embodied herein, and other compounds not specifically mentioned or described herein, in the preparation of a medicament.

These medicaments or formulations are useful in methods for the treatment of a disease such as peripheral neuropathy caused by physical injury or disease state, physical damage to the brain, physical damage to the spinal cord, stroke associated with brain damage, Huntington's Disease, Alzheimer's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, and Huntington's Disease. The present invention also relates to the use of carboxylic acid and carboxylic acid isostere compounds in methods for treating the above-mentioned neuropathies, neurological disorders, and neurological damage.

The present invention also relates to using the inventive compounds and compositions in the preparation of a medicament for the treatment of alopecia or promoting hair growth in an animal. The present invention also relates to the use in a method for treating alopecia or promoting hair growth in an animal, which comprises administering to said animal an effective amount of an N-heterocyclic carboxylic acid or carboxylic acid isostere.

The inventive method is particularly useful for treating male pattern alopecia, alopecia senilis, alopecia areata, alopecia resulting from skin lesions or tumors, alopecia resulting from cancer therapy such as

chemotherapy and radiation, and alopecia resulting from systematic disorders such as nutritional disorders and internal secretion disorders.

5 The present invention also relates to a method for treating a vision disorder, improving vision, treating memory impairment, or enhancing memory performance in an animal, which comprises administering to said animal an effective amount of N-heterocyclic carboxylic acid or carboxylic acid isostere. The present invention also
10 relates to using the inventive compounds and compositions in the preparation of a medicament for the treatment of a vision disorder, improving vision, treating memory impairment, or enhancing memory performance.

15 The inventive methods are particularly useful for treating various eye disorders including, but not limited to visual disorders, diseases, injuries, and complications, genetic disorders; disorders associated with aging or degenerative vision diseases; vision disorders correlating to physical injury to the eye, head,
20 or other parts of the body resulting from external forces; disorders resulting from environmental factors; disorders resulting from a broad range of diseases; and combinations of any of the above.

25 In particular, the compositions and methods of the present invention are useful for improving vision, or correcting, treating, or preventing visual (ocular) impairment or dysfunction of the visual system, including permanent and temporary visual impairment, without limitation. The present invention is also useful in
30 preventing and treating ophthalmologic diseases and disorders, treating damaged and injured eyes, and preventing and treating diseases, disorders, and injuries which result in vision deficiency, vision loss, or reduced capacity to see or process images, and the symptoms and
35 complications resulting from same. The eye diseases and

disorders which may be treated or prevented by the compositions and methods of the present invention are not limited with regard to the cause of said diseases or disorders. Accordingly, said compositions and methods are applicable whether the disease or disorder is caused by genetic or environmental factors, as well as any other influences. The compositions and methods of the present invention are particularly useful for eye problems or vision loss or deficiency associated with all of the following, without limitation: aging, cellular or physiological degeneration, central nervous system or neurological disorder, vascular defects, muscular defects, and exposure to adverse environmental conditions or substances.

The compositions and methods of the present invention are particularly useful in correcting, treating, or improving visual impairment, without limitation. Visual impairment in varying degrees occurs in the presence of a deviation from normal in one or more functions of the eye, including (1) visual acuity for objects at distance and near; (2) visual fields; and (3) ocular motility without diplopia. See *Physicians' Desk Reference (PDR) for Ophthalmology, 16th Edition, 6:47 (1988)*. Vision is imperfect without the coordinated function of all three. *Id.*

Said compositions and methods of use are also useful in correcting, treating, or improving other ocular functions including, without limitation, color perception, adaptation to light and dark, accommodation, metamorphopsia, and binocular vision. The compositions and methods of use are particularly useful in treating, correcting, or preventing ocular disturbances including, without limitation, paresis of accommodation, iridoplegia, entropion, ectropion, epiphora, lagophthalmos, scarring, vitreous opacities, non-reactive pupil, light scattering

disturbances of the cornea or other media, and permanent deformities of the orbit.

5 The compositions and methods of use of the present invention are also highly useful in improving vision and treating vision loss. Vision loss ranging from slight loss to absolute loss may be treated or prevented using said compositions and methods of use. Vision may be improved by the treatment of eye disorders, diseases, and injuries using the compositions and methods of the invention. However, improvements in vision using the compositions and methods of use are not so limited, and may occur in the absence of any such disorder, disease, or injury.

15 The compositions and methods of the present invention are also highly useful in preventing and/or treating sensorineural hearing loss in a patient. According to one aspect of the invention, methods are provided for treating damaged hair cells and auditory neurons.

20 It is further contemplated that administration of an inventive compound will protect hair cells and spiral ganglion neurons from traumatic damage, for example damage caused by noise trauma, acute or chronic treatment with cisplatin and aminoglycoside antibiotics or from damage resulting from a lack of neurotrophic factors resulting from interruption of transport of the factors from the axon to the cell body. Such treatment is expected to allow hair cells and/or auditory neurons to tolerate intermittent insults from either environmental noise trauma or treatment with ototoxins, and to slow down, prevent or reverse the progressive degeneration of the auditory neurons and hair cells which is responsible for hearing loss in pathological conditions such as presbycusis (age-related hearing loss), inherited sensorineural degeneration, and post-idiopathic hearing losses and to preserve the functional integrity of the

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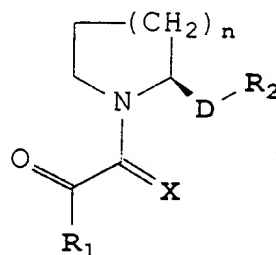
inner ear. Such treatment will also support the auditory neurons for better and longer performance of cochlear implants.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the severity of the particular disease or disorder being treated and form of administration.

Preferred Compounds of the Invention

Specific embodiments of the inventive compounds are presented in Tables I, II, and III. The present invention contemplates employing the compounds of Tables I, II and III, below, for use in compositions and methods to prevent and/or treat a neurological disorder in an animal, for use in compositions and methods to treat alopecia and promote hair growth in an animal, for use in compositions and methods to treat a vision disorder, improve vision, treat memory impairment, and enhance memory performance in an animal, and all the other uses suggested in this specification.

Table I

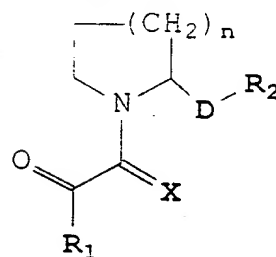


D is a bond and R₂ is COOH,
R₁

No.	X	n	R ₁
1	O	1	3,4,5-trimethylphenyl
2	O	2	3,4,5-trimethylphenyl
3	O	1	tert-butyl

	No.	X	n	R ₁
	4	O	3	tert-butyl
	5	O	1	cyclopentyl
5	6	O	2	cyclopentyl
	7	O	3	cyclopentyl
	8	O	1	cyclohexyl
	9	O	2	cyclohexyl
	10	O	3	cyclohexyl
10	11	O	1	cycloheptyl
	12	O	2	cycloheptyl
	13	O	3	cycloheptyl
	14	O	1	2-thienyl
	15	O	2	2-thienyl
15	16	O	3	2-thienyl
	17	O	1	2-furyl
	18	O	2	2-furyl
	19	O	3	2-furyl
	20	O	3	phenyl
20	21	O	1	1,1-dimethylpentyl
	22	O	2	1,1-dimethylhexyl
	23	O	3	ethyl

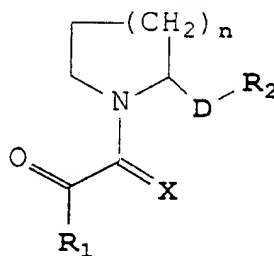
Table II



	No.	X	n	R ₁	D	R ₂
	24	S	1	1,1-dimethyl propyl	CH ₂	COOH
	25	S	1	1,1-dimethyl propyl	bond	COOH
30	26	O	1	1,1-dimethyl propyl	CH ₂	OH
	27	O	1	1,1-dimethyl propyl	bond	SO ₃ H
	28	O	1	1,1-dimethyl propyl	CH ₂	CN
	29	O	1	1,1-dimethyl propyl	bond	CN
	30	O	1	1,1-dimethyl propyl	bond	tetrazolyl
35	31	S	1	phenyl	(CH ₂) ₂	COOH
	32	S	1	phenyl	(CH ₂) ₃	COOH
	33	S	2	phenyl	CH ₂	COOH

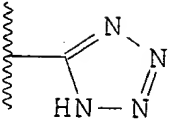
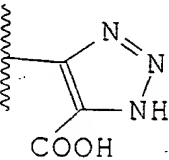
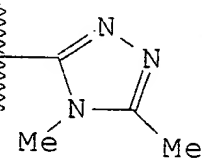
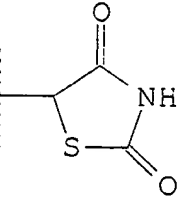
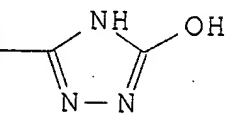
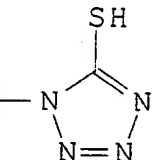
No.	X	n	R ₁	D	R ₂
34	O	1	1,1-dimethyl propyl	bond	CONH ₂
35	O	2	1,1-dimethyl propyl	bond	CONH ₂
36	S	2	2-furyl	bond	PO ₃ H ₂
37	O	2	propyl	(CH ₂) ₂	COOH
38	O	1	propyl	(CH ₂) ₃	COOH
39	O	1	tert-butyl	(CH ₂) ₄	COOH
40	O	1	methyl	(CH ₂) ₅	COOH
41	O	2	phenyl	(CH ₂) ₆	COOH
42	O	2	3,4,5- trimethoxy-phenyl	CH ₂	COOH
43	O	2	3,4,5- trimethoxy-phenyl	CH ₂	tetrazolyl

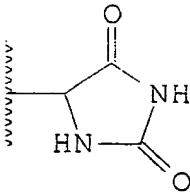
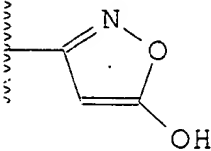
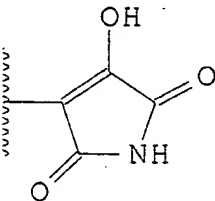
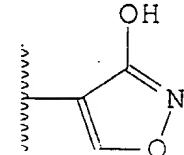
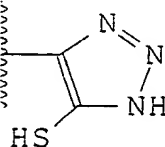
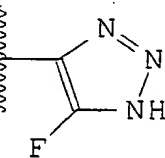
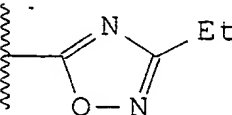
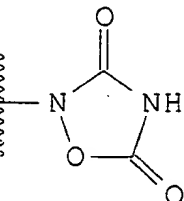
TABLE III

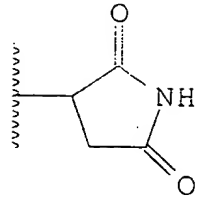
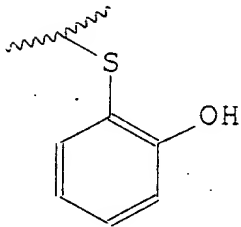
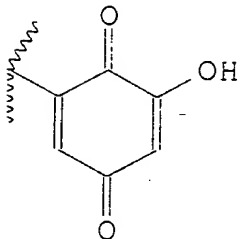
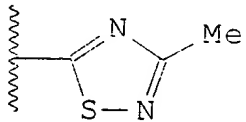
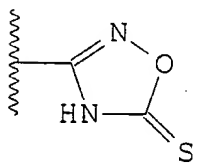


No.	n	X	D	R ₂	R ₁
44	1	S	bond	COOH	Phenyl
45	1	O	bond	COOH	α-MethylBenzyl
46	2	O	bond	COOH	4-MethylBenzyl
47	1	O	bond	Tetrazole	Benzyl
48	1	O	bond	SO ₃ H	α-MethylBenzyl
49	1	O	CH ₂	COOH	4-MethylBenzyl
50	1	O	bond	SO ₂ HNMe	Benzyl
51	1	O	bond	CN	α-MethylBenzyl
52	1	O	bond	PO ₃ H ₂	4-MethylBenzyl
53	2	O	bond	COOH	Benzyl
54	2	O	bond	COOH	α-MethylBenzyl
55	2	O	bond	COOH	4-MethylBenzyl
56	2	S	bond	COOH	3,4,5-trimethoxyphenyl
57	2	O	bond	COOH	Cyclohexyl

	No.	n	X	D	R ₂	R ₁
	58	2	O	bond	PO ₂ HET	i-propyl
	59	2	O	bond	PO ₃ HPropyl	ethyl
5	60	2	O	bond	PO ₃ (Et) ₂	Methyl
	61	2	O	bond	OMe	tert-butyl
	62	1	O	bond	OEt	n-pentyl
	63	2	O	bond	OPropyl	n-hexyl
	64	1	O	bond	OBUTYL	Cyclohexyl
10	65	1	O	bond	OPentyl	cyclopentyl
	66	1	O	bond	OHexyl	n-heptyl
	67	1	O	bond	SMe	n-octyl
	68	1	O	bond	SEt	n-nonyl
	69	2	O	bond	SPropyl	2-indolyl
15	70	2	O	bond	SButyl	2-furyl
	71	2	O	bond	NHCOMe	2-thiazolyl
	72	2	O	bond	NHCOEt	2-thienyl
	73	1	O	CH ₂	N(Me) ₂	2-pyridyl
	74	1	O	(CH ₂) ₂	N(Me)Et	1,1-dimethylpropyl
20	75	1	O	(CH ₂) ₃	CON(Me) ₂	1,1-dimethylpropyl
	76	1	O	(CH ₂) ₄	CONHMe	1,1-dimethylpropyl
	77	1	O	(CH ₂) ₅	CONHEt	1,1-dimethylpropyl
	78	1	O	(CH ₂) ₆	CONHPropyl	1,1-
	79	1	O	bond		dimethylpropyl
25	80	1	O	bond	CONH(O)Me	Benzyl
	81	1	O	bond	CONH(O)Et	α-Methylphenyl
	82	1	O	bond	CONH(O)Propyl	4-Methylphenyl
	83	1	O	(CH ₂) ₂	COOH	Benzyl
	84	1	O	bond	COOH	α-Methylphenyl
30	85	1	O	bond	COOH	4-Methylphenyl
	86	1	O	CH ₂	COOH	1,1-dimethylpropyl
	87	1	O	(CH ₂) ₂	COOH	1,1-dimethylbutyl
	88	1	O	(CH ₂) ₃	COOH	1,1-dimethylpentyl
	89	1	O	(CH ₂) ₄	COOH	1,1-dimethylhexyl
35	90	1	O	(CH ₂) ₅	COOH	1,1-dimethylethyl
				(CH ₂) ₆	COOH	iso-propyl
	91	1	O	(CH ₂) ₇	COOH	tert-butyl
	92	1	O	(CH ₂) ₈	COOH	1,1-dimethylpropyl
40	93	1	O	(CH ₂) ₉	COOH	benzyl
	94	1	O	(CH ₂) ₁₀	COOH	1,1-dimethylpropyl
	95	1	O	C ₂ H ₂	COOH	cyclohexylmethyl
45	96	1	O	2-OH, Et	COOH	1,1-dimethylpropyl
	97	1	O	2-butylene	COOH	1,1-dimethylpropyl
	98	1	S	i-Pro	COOH	1,1-dimethylpropyl
	99	2	S	tert-Bu	COOH	phenyl
50	100	2	O	2-nitro-hexyl	COOH	1,1-dimethylpropyl
	101	1	O	(CH ₂) ₂	CN	1,1-dimethylpropyl
	102	1	O	(CH ₂) ₃	CN	1,1-dimethylpropyl

	No.	n	X	D	R2	R1
5	103	3	O	bond	CONHNHSO ₂ Me	Benzyl
	104	3	O	bond	CONHNHSO ₂ Et	α-Methylphenyl
	105	3	O	bond	CONHSO ₂ Me	4-Methylphenyl
	106	1	O	bond	CONHNHSO ₂ Et	Phenyl
	107	2	O	bond	CON (Me) CN	α-Methylphenyl
10	108	1	O	bond	CON (Et) CN	4-Methylphenyl
	109	1	O	(CH ₂) ₂	COOH	methyl
	110	1	O	(CH ₂) ₃	COOH	ethyl
	111	1	O	(CH ₂) ₄	COOH	n-propyl
	112	1	O	(CH ₂) ₅	COOH	t-butyl
15	113	1	O	(CH ₂) ₆	COOH	Pentyl
	114	1	O	(CH ₂) ₇	COOH	Hexyl
	115	1	O	(CH ₂) ₈	COOH	Septyl
	116	1	O	(CH ₂) ₉	COOH	Octyl
	117	1	O	C ₂ H ₂	COOH	Cyclohexyl
20	118	2	O	bond		1,1-dimethylpropyl
	119	1	O	bond		1,1-dimethylpropyl
	120	1	O	bond		1,1-dimethylpropyl
	121	1	O	bond		1,1-dimethylpropyl
	122	1	O	bond		1,1-dimethylpropyl
25	123	1	O	bond		1,1-dimethylpropyl

No.	n	X	D	R2	R1
124	1	O	bond		1,1-dimethylpropyl
125	1	O	bond		1,1-dimethylpropyl
126	1	O	bond		1,1-dimethylpropyl
127	1	O	bond		1,1-dimethylpropyl
128	1	O	bond		1,1-dimethylpropyl
129	1	O	bond		1,1-dimethylpropyl
130	1	O	bond		1,1-dimethylpropyl
131	1	O	bond		1,1-dimethylpropyl

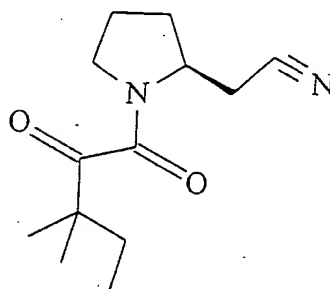
No.	n	X	D	R2	R1
132	1	O	bond		1,1-dimethylpropyl
133	1	O	bond		1,1-dimethylpropyl
134	1	O	bond		1,1-dimethylpropyl
135	1	O	bond		1,1-dimethylpropyl
136	1	O	bond		1,1-dimethylpropyl
137	1	O	bond	COOH	1,1-dimethylpropyl
138	2	O	bond	COOH	1,1-dimethylpropyl

Specific embodiments of the present invention may be found in TABLE IV below:

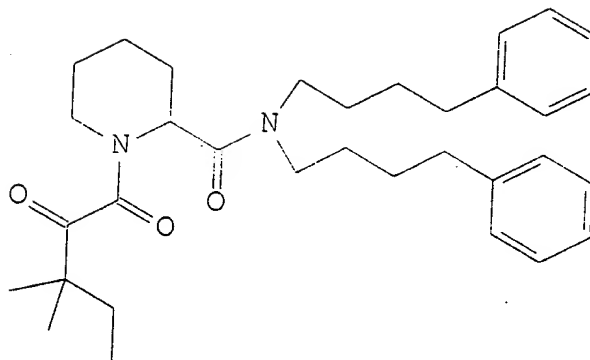
TABLE IV

Compound No.Compound Structure

28

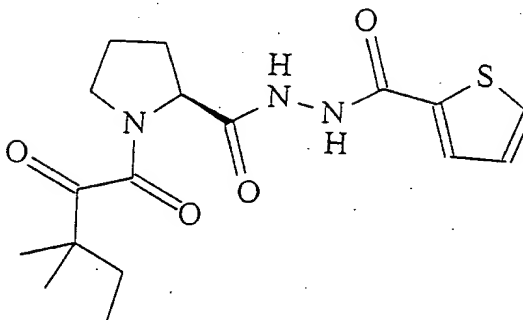


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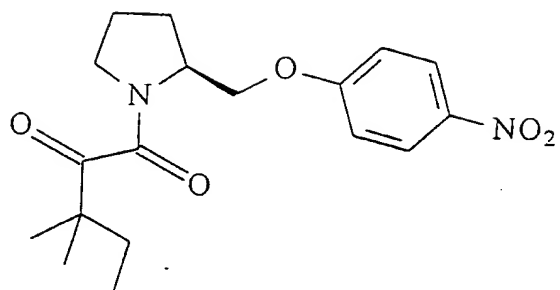


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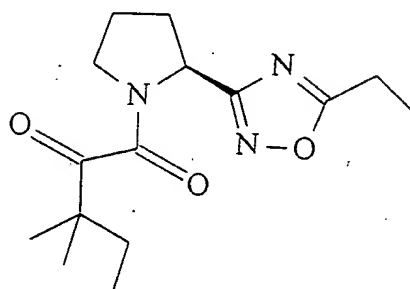
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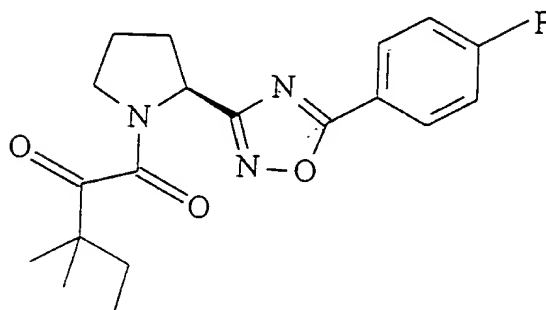
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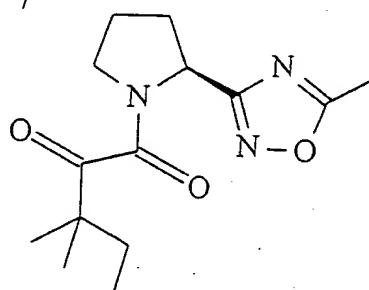
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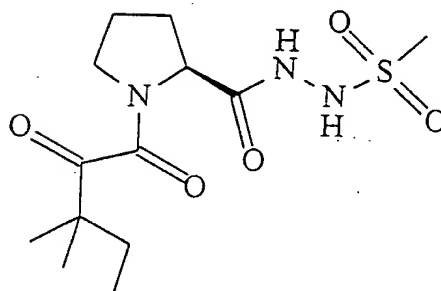
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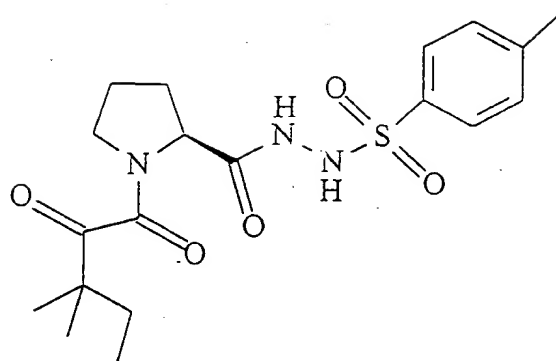
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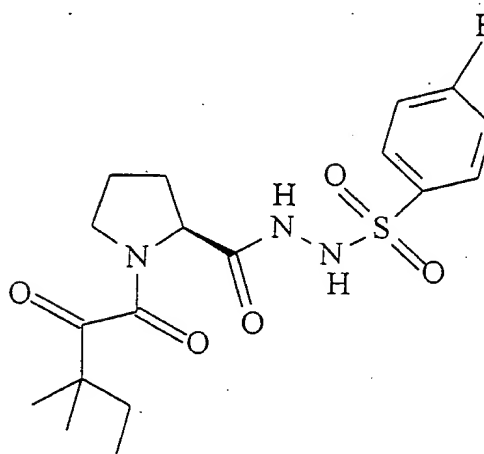
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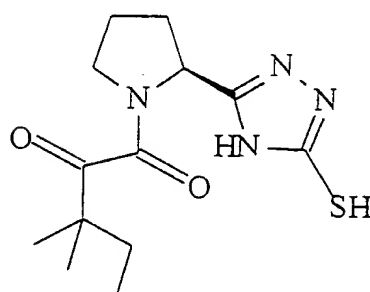
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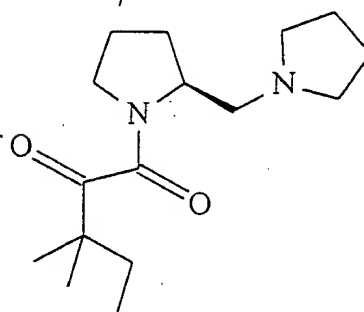
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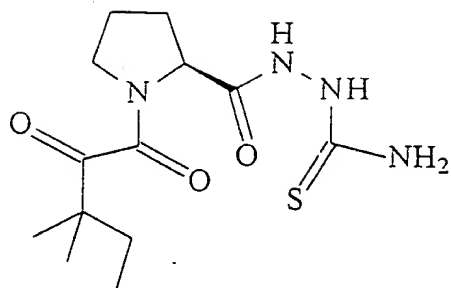
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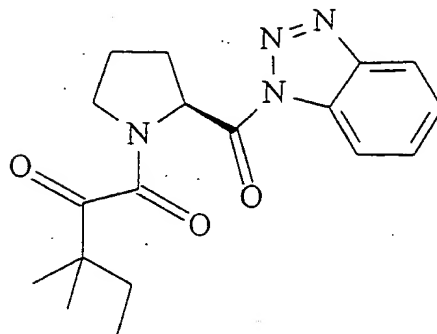
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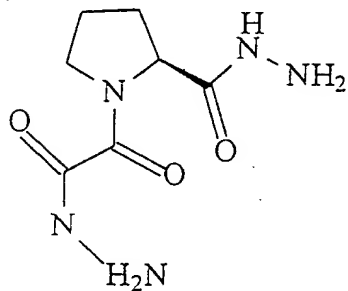
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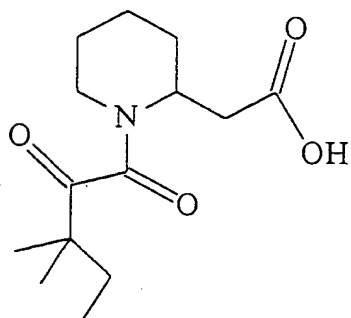


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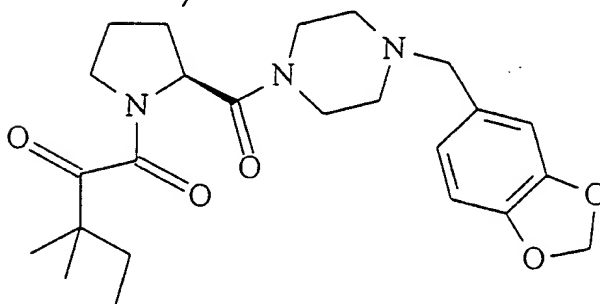


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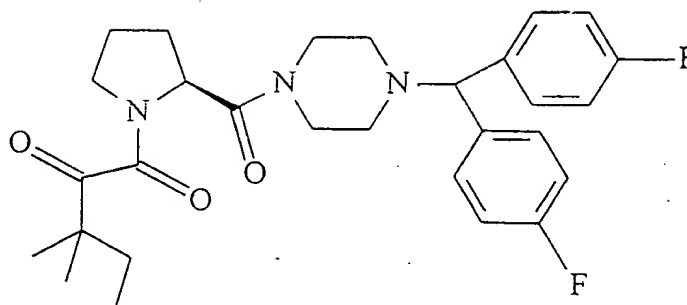
153



154



155



5 The following examples are illustrative of preferred
embodiments of the invention and are not to be construed
as limiting the invention thereto. All polymer molecular
weights are mean average molecular weights. All
percentages are based on the percent by weight of the
10 final delivery system or formulation prepared unless
otherwise indicated and all totals equal 100% by weight.

Other compounds which are carboxylic acids and
isosteres of N-heterocyclic compounds within the scope of
the present invention are those compounds which may
15 possess immunosuppressive, non-immunosuppressive, or other
activities as long as they also are useful in preventing
and/or treating neurological disorders, including
physically damaged nerves and neurodegenerative diseases;
in treating alopecia and promoting hair growth; in
20 treating vision disorders and/or improving vision; and in
treating memory impairment and/or enhancing memory
performance.

MPTP Model of Parkinson's Disease in Mice

MPTP lesioning of dopaminergic neurons in mice was
25 used as an animal model of Parkinson's Disease. Four week
old male CD1 white mice were dosed i.p. with 30 mg/kg of
MPTP for 5 days. The inventive compounds (4 mg/kg), or
vehicle, were administered s.c. along with the MPTP for 5
days, as well as for an additional 5 days following
30 cessation of MPTP treatment. At 18 days following MPTP

5 treatment, the animals were sacrificed and the striata
were dissected and homogenized. Immunostaining was
performed on saggital and coronal brain sections using
anti-tyrosine hydroxylase Ig to quantitate survival and
10 recovery of dopaminergic neurons. In animals treated with
MPTP and vehicle, a substantial loss of functional
dopaminergic terminals was observed as compared to non-
lesioned animals. In another protocol, test compounds
were administered only subsequent to MPTP-induced
15 lesioning. Thus, after animals were treated with MPTP for
5 days, an additional 3 days passed before beginning oral
drug treatment on day 8. Animals were treated with the
inventive compounds (0.4 mg/kg), administered orally, once
a day for 5 days total. On day 18, the animals were
sacrificed and analyzed as described above.

Table V presents the percent recovery of dopaminergic
neurons in the first (concurrent dosing) paradigm in
animals receiving carboxylic acid or carboxylic acid
isostere compounds of the present invention.

20 Table V, below, shows the remarkable
neuroregenerative effects of the inventive carboxylic acid
or carboxylic acid isostere related compounds illustrating
the neurotrophic capability of carboxylic acid isosteres
as a class showing that lesioned animals receiving the
25 carboxylic acid or carboxylic acid isostere compounds
provide a remarkable recovery of TH-stained dopaminergic
neurons.

Additional claimed or comparative carboxylic acids
and isosteres of N-heterocyclic compounds which also show
30 the remarkable neurotrophic and hair growth effects of the
present invention are shown below in Table V:

Table V - MPTP Neurodegenerative Model

	<u>COMPOUND</u>	Post-MPTP % TH RECOVERY <u>10mg/kg p.o.</u>
5	Compound 26	23.2
	Compound 28	15.7
	Compound 29	34.1
	Compound 30	19.6
	Compound 35	46.5
10	Compound 137	26.7
	Compound 140	10.4
	Compound 141	26.3
	Compound 143	29.2
	Compound 144	41.7
15	Compound 146	40.6
	Compound 147	n/a
	Compound 148	21.4

Percent striatal innervation density was quantitated in brain sections with an anti-tyrosine hydroxylase immunoglobulin, which is indicative of functional dopaminergic neurons. The striatal innervation density of 23% for animals pretreated with only a vehicle and administered a vehicle orally during treatment, is indicative of normal non-lesioned striatal tissue. Striatal innervation density is reduced to 5% for animals pretreated with MPTP and administered a vehicle orally during treatment, and is indicative of MPTP-induced lesioning. Surprisingly, striatal innervation density is increased 8-13% for animals pretreated with MPTP and administered 0.4 mg/kg of an inventive compound orally during treatment, indicating substantial neuronal regeneration after induction of MPTP-derived lesions.

In Vivo Hair Generation Test With C57 Black 6 Mice

C57 black 6 mice were used to demonstrate the hair revitalizing properties of the N-heterocyclic carboxylic

acids or carboxylic acid isosteres. Referring now to
FIGS. 1 and 2 of the drawings, C57 black 6 mice,
approximately 7 weeks old, had an area of about 2 inches
by 2 inches on their hindquarters shaved to remove all
existing hair. Care was taken not to nick or cause
abrasion to the underlaying dermal layers. The animals
were in anagen growth phase, as indicated by the pinkish
color of the skin. Referring now to FIG. 2, four animals
per group were treated by topical administration with 20%
propylene glycol vehicle (FIG. 2), or neuroimmunophilin
FKBP ligands dissolved in the vehicle. The animals were
treated with vehicle or neuroimmunophilin ligands every 48
hours (3 applications total over the course of 5 days) and
the hair growth was allowed to proceed for 6 weeks. Hair
growth was quantitated by the percent of shaved area
covered by new hair growth during this time period.

FIG. 2 shows that animals treated with vehicle
exhibited only a small amount of hair growth in patches or
tufts, with less than 3% of the shaved area covered with
new growth.

In contrast, FIG. 3 shows that animals treated for 2
weeks with the N-heterocyclic carboxylic acid compounds
i.e. compound A (137), compound B (138), and compound G
(35) exhibited dramatic hair growth, covering greater than
25% of the shaved area in all animals for two of the
compounds.

FIG. 3 shows the relative hair growth on shaven C57
black 6 mice 14 days after being treated with one of three
N-heterocyclic carboxylic acids or carboxylic acid
isosteres. The mice had a 2 x 2 inch region on their
backside shaved to remove all hair. Care was taken not to
nick or cause abrasion to the underlying dermal layers.
Compounds at a concentration of 1 μ mole per milliliter
were carefully applied to the shaved area of the mice (5
mice per group) three times per week. Hair growth was

- evaluated 14 days after initiation of drug treatment. The relative scale for assessing hair growth is as follows:
- 0 = no growth;
 - 1 = beginning of growth in small tufts;
 - 5 2 = hair growth covering over <25% of shaved area;
 - 3 = hair growth covering over >25% of shaved area, but less than 50% of shaved area;
 - 4 = hair growth covering over >50% of shaved area, but less than 75% of shaved area;
 - 10 5 = complete hair growth of shaved area.

Retinal Ganglion Cell Survival and Arrest of Axonal Dying
Back Following Optic Nerve Transection

15 Transection of the mammalian optic nerve results in a brief period of abortive regeneration, but the majority of axotomized neurons die and the axons from many persisting ganglion cells die back beyond the optic nerve head. The present Example was designed to examine the
20 neuroprotective effects of GPI-1046 following optic nerve transection.

Retinal ganglion cells in adult male Sprague Dawley rats were retrogradely labeled by fluorogold injection in the LGNd and four days later the optic nerves were
25 transected 5 mm behind the globe. Groups of animals received either GPI-1046 10mg/kg/day s.c. or vehicle for 28 days. All experimental animals and controls were sacrificed 90 days after transection.

By 90 days only - 10% of the FG labeled ganglion cell
30 population survived but less than half of these neurons maintained axons that extended past the optic nerve head, as detected with RT97 neurofilament immunohistochemistry. GPI-1046 treatment produced a moderate degree of perikaryal neuroprotection, sparing 25% of the ganglion
35 cell population, and preserved the axons of virtually all

protected neurons in the proximal stump of the transected nerve. These results indicate that treatment with the FKBP neuroimmunophilin ligand GPI-1046 produces a fundamental alteration in the pathological process following injury to CNS tracts.

These results also demonstrate that the small molecule FKBP neuroimmunophilin ligand GPI 1046 enhances neurite outgrowth in culture, enhance peripheral nerve regeneration, and stimulate sprouting within the CNS following partial deafferentation.

In Vivo Retinal Ganglion Cell and Optic Nerve Axon Tests

The extent of degeneration reduction or prevention in retinal ganglion cells and optic nerve axons was determined in a vision loss model utilizing surgical optic nerve transection to simulate mechanical damage to the optic nerve. The effects of several N-heterocyclic derivative neuroimmunophilin FKBP ligands on retinal ganglion cells neuroprotection and optic nerve axon density was determined experimentally, comparing 14 day and 28 day N-heterocyclic derivative neuroimmunophilin FKBP ligand treatments. The effects of treatment with N-heterocyclic derivative neuroimmunophilin FKBP ligands on retinal ganglion cells and optic nerve axons was correlated.

Surgical Procedures

Adult male Sprague Dawley rats (3 months old, 225-250 grams) were anesthetized with a ketamine (87mg/kg) and xylazine (13mg/kg) mixture. Retinal ganglion cells were pre-labeled by bilateral stereotaxic injection of the fluorescent retrogradely transported marker fluoro-gold (FG, 0.5 microliters of 2.5% solution in saline) at the coordinates of the LGNd (4.5 millimeters post β , 3.5 millimeters lateral, 4.6 millimeters below dura). Four days later, FG labeled rats underwent a second surgery for

microsurgical bilateral intraorbital optic nerve transection 4-5 millimeters behind the orbit.

Experimental animals were divided into six experimental groups of six rats (12 eyes) per group. One group received an N-heterocyclic derivative neuroimmunophilin FKBP ligand (10 milligrams per kg per day sc in PEG vehicle (20 percent propylene glycol, 20 percent ethanol, and 60 percent saline)) for 14 days. A second group received the same N-heterocyclic derivative neuroimmunophilin FKBP ligand dose for 28 days. Each treated group had a corresponding sham/surgery and transection control group which received corresponding 14 or 28 day dosing with the vehicle only.

All animals were sacrificed 90 days after optic nerve transection and perfused pericardially with formalin. All eyes and optic nerves stumps were removed. Cases were excluded from the study if the optic nerve vasculature was damaged or if FG labeling was absent in the retina.

Retinal Ganglion Cell Counts

Retinas were removed from eyes and prepared for wholemount analysis. For each group, five eyes with dense and intense FG labeling were selected for quantitative analysis using a 20 power objective. Digital images were obtained from five fields in the central retina (3-4 millimeters radial to optic nerve head). FG labeled Large (>18 μ m), medium (12-16 μ m), and small (<10 μ m) ganglion cells and microglia were counted in five 400 μ m by 400 μ m fields per case, 5 cases per group.

Examination of Optic Nerves

Proximal and distal optic nerve stumps were identified, measured, and transferred to 30% sucrose saline. The proximal stumps of five nerves were blocked and affixed to a chuck, and 10 micron cross sections were cut on a cryostat; one in ten sections were saved per set. Sections including the region 1-2 mm behind the orbit were

reacted for RT97 neurofilament immunohistochemistry. Analysis of optic nerve axon density was performed using a 63 power oil immersion lens, a Dage 81 camera, and the Simple Image Analysis program. RT97 positive optic nerve axons were counted in three 200 μ m by 200 μ m fields per nerve. The area of the nerve was also determined for each case at 10 power.

The 14 day course of treatment with an N-heterocyclic derivative neuroimmunophilin FKBP ligand provided moderate neuroprotection of retinal ganglion cells observed 28 days after optic nerve transection. However, by 90 days after transection, only 5% of the ganglion cell population remained viable.

90 days after optic nerve transection the number of axons persisting in the proximal stump of the optic nerve represented approximately one half of the number of surviving ganglion cells in groups of animals that received vehicle alone or the 14 day course of treatment with an N-heterocyclic derivative neuroimmunophilin FKBP ligand. These results indicate that over half of the transected ganglion cell axons retract beyond the optic nerve head, and that treatment with an N-heterocyclic derivative neuroimmunophilin FKBP ligand during the first 14 days after optic nerve transection is not sufficient to arrest this retraction.

More prolonged treatment with an N-heterocyclic derivative neuroimmunophilin FKBP ligand during the 28 day course of treatment produced a moderate increase in retinal ganglion cell neuroprotection. Approximately 12% of the vulnerable retinal ganglion cell population was protected. A similar proportion (~50%) of optic nerve axon density sparing was also observed. These results demonstrate the startling result that extending the duration of treatment with an N-heterocyclic derivative neuroimmunophilin FKBP ligand to 28 days after transection

completely arrests the regression of damaged axons for essentially the entire surviving population of retinal ganglion cells.

5

Figure 4. GPI 1046 protects retinal ganglion cells against degeneration following retinal ischemia.

Retinal ganglion cells were retrogradely labeled in adult rats by bilateral injection of fluorogold in their lateral geniculate nuclei. Labeled ganglion cells in the normal rat retina appear as white profiles against the dark background (Figure 4A). Complete retinal ischemia was produced by infusing normal saline solution into the retinal vitreous cavity of each eye until the intraocular pressure exceeded arterial blood pressure. 28 days after the ischemic episode extensive degeneration of retinal ganglion cell was evidenced by massive reduction in the density of fluorogold labeled cells (Figure 4B). Administration of GPI 1046 (10mg/kg, s.c.) 1 hour prior to the ischemic episode and at 10mg/kg/day for the next four days produced noticeable protection of a large proportion of the vulnerable ganglion cell population (Figure 4C).

Figure 5. GPI 1046 prevents degeneration of optic nerve axons and myelin following retinal ischemia

Examination of the optic nerves from the same retinal ischemia cases reveals that GPI 1046 produces dramatic protection of optic nerve element from ischemic degeneration. Toluidine blue staining of epon embedded optic nerve cross sections revealed the detail of myelin sheaths (white circles) and optic nerve axons (black centers) in the normal rat optic nerve. Optic nerves from vehicle treated cases examined 28 days after a 1 hour retinal ischemic episode are characterized by a decreased density of optic nerve axons and the appearance of

numerous degenerating myelin figures (bright white filled circles). Treatment with GPI 1046 protected the majority of optic nerve axons from degeneration and also dramatically decreased the density of degenerating myelin figures.

Figure 6. GPI 1046 provides moderate protection against retinal ganglion cell death after optic nerve transection

Complete transection of the optic nerve 5 mm from the eyeball produces massive degeneration of retinal ganglion cells, representing loss of >87% of the normal ganglion cell population 90 days after the injury. Few spared fluorogold pre labeled ganglion cells are present in vehicle treated cases (large white figures) among a population of small microglia that digest the debris of the degenerating cells and take up the fluorogold label (Figure 6A). Treatment with GPI 1046 for 14 days resulted in a small but not significant increase in the density of retinal ganglion cells that survived 90 days after transection but treatment with GPI 1046 for the first 28 days after transection produced moderate but significant protection of 12.6% of the vulnerable ganglion cell population (Figure 6B).

Figure 7. GPI 1046 treatment duration significantly affects the process of optic nerve axonal degeneration after transection.

Examination of optic nerve axon density in the proximal stump of the optic nerve from the same cases revealed a more dramatic protection afforded by GPI 1046 treatment. 90 days after transection few ganglion cell axons remain within the optic nerve (Figure 7B), representing only 5.6% of the normal population. The loss of axons reflects both the death of retinal ganglion cells

and the regression or "dying back" of the axons of ~ 70% of the small surviving ganglion cell population into the retina itself (Table 1). Treatment with GPI 1046 for the first 14 days after optic nerve transection produced a small but significant 5.3% protection of optic nerve axons (Figure 7D, Table 1), but treatment with the same dose of GPI 1046 for 28 days resulted in the protection of optic nerve axons for the vast majority (81.4%) of spared retinal ganglion cells (Figure 7C, Table 1).

Figure 8. GPI 1046 treatment produces a greater effect on optic nerve axons than ganglion cell bodies

This summary figure shows data from Figure 6 ganglion cell protection and higher power photomicrographs of optic nerve axon protection (Figure 8A&B, upper panels). 28 day treatment with GPI 1046 produced a significant increase in the density of large, and particularly medium and small caliber optic nerve axons (Figure 8C&D, lower panels).

Figure 9. GPI 1046 treatment for 28 days after optic nerve transection prevents myelin degeneration in the proximal stump

Myelin basic protein immunohistochemistry labels fascicles (darker labeled 'islands') of myelinated axons in the normal optic nerve (Figure 9A, upper left). 90 days after transection extensive degeneration of myelin is evident in vehicle treated cases, characterized by the loss of fascicular organization and the appearance of numerous large dense degenerating myelin figures (Figure 9B, upper right). Treatment with GPI 1046 for the first 14 days after optic nerve transection did not alter the pattern of myelin degeneration (Figure 9C, lower left panel), and yielded an insignificant 1.6% quantitative recovery in myelin density (Table 1). Extending the GPI

1046 treatment course through the first 28 days after optic nerve transection produced a dramatic preservation of the fascicular staining pattern for myelin basic protein in the proximal stump of the optic nerve and
5 decreased the density of degenerating myelin figures (Figure 9D, lower right panel), representing a '70% recovery of myelin density (Table 1).

Figure 10. FKBP-12 immunohistochemistry labels
10 oligodendroglia (large dark cells with fibrous processes), the cells which produce myelin, located between the fascicles of optic nerve fibers, and also some optic nerve axons.

15 Figure 11. GPI 1046 treatment for 28 days after optic nerve transection prevents myelin degeneration in the distal stump.

Complete transection of the optic nerve leads to degeneration of the distal segments (axon fragments disconnected from the ganglion cell bodies), and the
20 degeneration of their myelin sheaths. 90 days after transection (Figure 11B) myelin basic protein immunohistochemistry reveals the near total loss of fascicular organization (present in the normal optic nerve, Figure 11A) and the presence of numerous dense
25 degenerating myelin figures. Quantitation reveals that the cross sectional area of the transected distal stump shrinks by 31% and loses approximately 1/2 of its myelin (Table 1). Treatment with GPI 1046 for the first 14 days
30 after transection did not protect against shrinkage of the distal stump but did slightly increase the density of myelin, though the density of degenerating myelin figures remained high (Figure 11C, Table 1). GPI 1046 treatment through the first 28 days produced dramatic protection of

the fascicular pattern of myelin labeling, decreased the density of degenerating myelin figures, prevented cross sectional shrinkage of the distal stump of the transected nerve and maintained the myelin levels at ~99% of normal levels (Figure 11D, Table 1).

Figure 12. 28 day treatment with GPI 1046 treatment beginning 8 weeks after onset of streptozotocin induced diabetes decreases the extent of neovascularization in the inner and outer retina and protects neurons in the inner nuclear layer (INL) and ganglion cell layer (GCL) from degeneration.

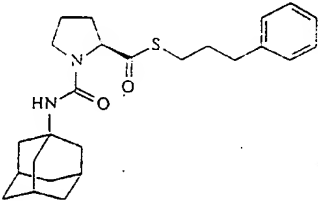
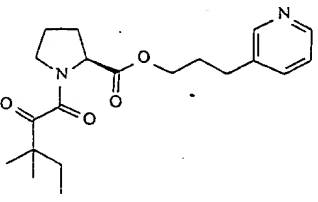
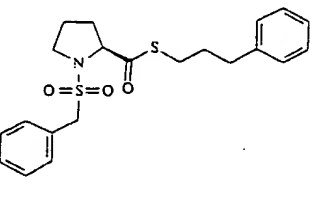
-- Negative images of cresyl violet stained tangential retinal sections reveals perikarya in the three cellular layers (Figure 12A). The retinae of streptozotocin treated animals administered only vehicle (Figure 12B) exhibited loss of cells from the ONL and INL, decreased thickness of the Outer plexiform layer (the dark area between ONL and INL) and a dramatic increase in the size and density of retinal blood vessels (large black circular outlines) in the INL, OPL, ONL and the photoreceptor layer (PR, the gray fuzzy area above the ONL). GPI 1046 treatment reduced neovascularization (i.e. prevented the proliferation of blood vessels) in the PR, ONL, OPL and INL. Although GPI 1046 did not appear to protect against neuronal loss in the ONL, it appeared to decrease the loss of neurons in both the INL and GCL compared to streptozotocin/vehicle treated controls.

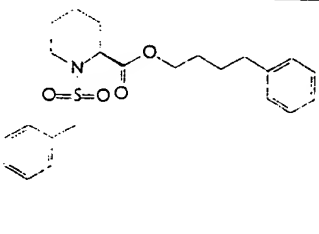
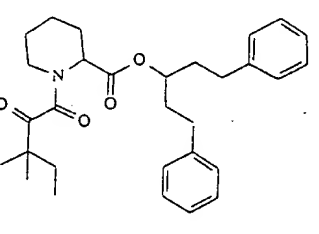
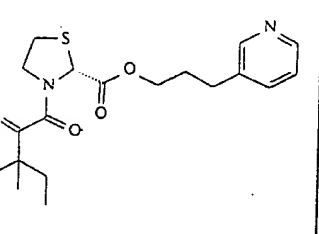
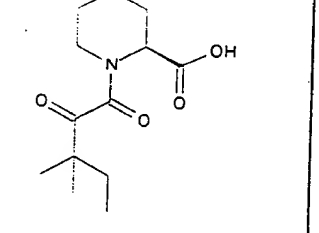
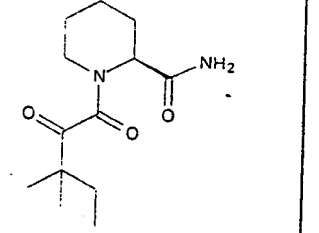
Protection of retinal ganglion cell axons from degeneration following optic nerve transection

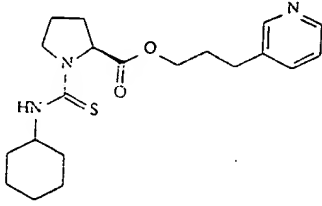
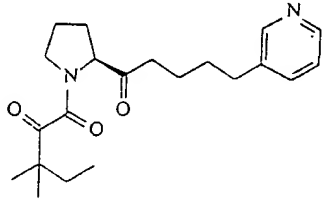
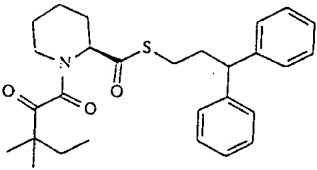
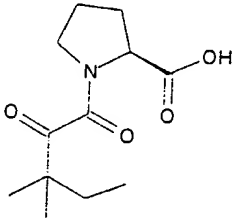
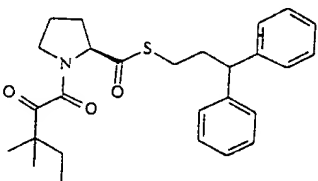
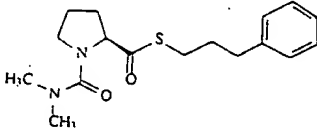
Efficacy of representative compounds from different immunophilin ligand series in protecting retinal ganglion cell axons from degeneration following optic nerve transection is set forth in Table VI.

TABLE VI

Efficacy of Representative Compounds from Different Immunophilin Ligand
Series in Protecting Retinal Ganglion
Cell Axons from Degeneration Following
Optic Nerve Transection

Compound	Structure	Comments	RT97+RG C axon density 14 days after ON transection (% ON axons rescued)
B		Adamantyl Thioester of Urea K_i Rotamase = 149 nM Clearance = ? μ l/min.	100 % ± 5.2 % SEM
A (GPI 1046)		Ester K_i Rotamase = 7.5 nM Clearance = 63.8 μ l/min.	60.5 % ± 3.9 % SEM
C		Sulfonamide K_i Rotamase = 107 nM Clearance = 31.1 μ l/min.	60.4 % ± 3.1 % SEM

D		Pipecolic Sulfonamide K_i Rotamase = ? nM Clearance = ? μ l/min.	58.4 % $\pm 6.4\%$ SEM
E		Ester of Pipecolic Acid K_i Rotamase = 20 nM Clearance = 41.8 μ l/min.	56.6 % $\pm 9.4\%$ SEM
F		Proline heterocycle K_i Rotamase = 272 nM Clearance = ? μ l/min.	55.1 % $\pm 5.9\%$ SEM
G		Pipecolic acid dimethyl ketone K_i Rotamase > 10,000 nM Clearance = ? μ l/min.	34.0 % $\pm 4.8\%$ SEM
H		Pipecolic acid dimethyl amide K_i Rotamase = ? nM Clearance = ? μ l/min.	30.3 % $\pm 8.0\%$ SEM

I		Ester of thiourea K_i Rotamase = 131 nM Clearance = 8.0 μ l/min.	23.8% $\pm 5.3\%$ SEM
J		Ketone analog of GPI 1046 K_i Rotamase = 210 nM Clearance = 1.5 μ l/min.	15.8% $\pm 4.8\%$ SEM
K		Pipecolic acid thioester K_i Rotamase = 86 nM Clearance = 4.5 μ l/min.	13.0% $\pm 4.2\%$ SEM
L		Prolyl acid K_i Rotamase > 7743 nM Clearance = 5.2 μ l/min.	7.8% $\pm 3.0\%$ SEM
M		Thioester K_i Rotamase = 7 nM Clearance = 12.5 μ l/min.	-6.3% $\pm 3.9\%$ SEM
N		K_i Rotamase = 722 nM Clearance = 21.9 μ l/min.	ND

Morris Watermaze/Aging and Memory Testing

5 Aged rodents exhibit marked individual differences in performance on a variety of behavioral tasks, including two-choice spatial discrimination in a modified T-maze, spatial discrimination in a circular platform task, passive avoidance, radial maze tasks, and spatial navigation in a water pool.

10 In all of these tasks, a proportion of aged rats or mice perform as well as the vast majority of young control animals, while other animals display severe impairments in memory function compared to young animals. For example, Fischer and colleagues showed that the proportion of rats displaying significant impairments in spatial navigation
15 increases with age, (Fischer et al. 1991b) with 8% of all 12 month old, 45% of 18 month old, 53% of 24 month old, and 90% of all 30 month old rats displaying impairments in spatial acquisition of the Morris watermaze task relative to young controls.

20 Specifically, rodent spatial learning and memory decline during aging has been accepted by many investigators as an intriguing correlative animal model of human senile dementia. Cholinergic function in the hippocampus has been extensively studied as a component of
25 spatial learning in rodents, and declining hippocampal cholinergic function has been noted in parallel with the development of learning and memory impairments. In addition, other neurotransmitter systems have been shown to contribute to spatial learning, and to decline with
30 age, such as the dopaminergic and noradrenergic, serotonergic, and glutamatergic systems.

Also, reports on age-related deficits of hippocampal long-term potentiation (LTP)-induction, a reduction in theta rhythm frequency, a loss of experience-dependent
35 plasticity of hippocampal place-units, and reductions in

hippocampal protein kinase C are in keeping with the concept that no single underlying pathology can be identified as the cause of age-related behavioral impairment in rodents. However, the various experimental therapeutic approaches that have been undertaken to improve memory function in aged rodents have been somewhat slanted towards the cholinergic hypothesis.

The Morris watermaze is widely used for assessing spatial memory formation and retention in experimental animals. The test depends on the animal's ability to utilize spatial visual information in order to locate a submerged escape platform in a water tank. It is important that the tank itself be as devoid of specific visual features as possible - thus, it is always circular in shape, the sides are kept smooth and in uniform dull colors, and the water is rendered opaque with nontoxic watercolour pigment or powdered milk. This is to ensure that the animal navigates only by the use of more distant visual cues, or by the use of intra-maze cues specifically provided by the experimenter.

The tank is filled to a level which forces the animal to swim actively. Normal mice and rats react aversively to the swimming part of the test and will climb onto, and remain on, an escape platform from which they are removed to a heated resting cage.

If the platform is visible (i.e. above the surface), animals placed in the tank will quickly learn to home in on the platform and climb out onto it. Testing with a visible platform will also ensure that the experimental animals are not blind and show sufficient motivation and stamina to perform the task, which can be important in experiments involving aged rodents. If the platform is invisible (i.e. submerged just below the surface), normal animals learn to use distant visual cues in the test room for orientation in the test tank, and, when placed in the

tank, will quickly home in on the approximate location of the platform and circle in that area until the platform is found.

5 The animals' path, speed, and swim time are tracked with a ceiling camera for later computerized analysis. Over the course of several successive trials, spatial learning can therefore be defined as a drop of distance swum, or time elapsed, from placement in the tank until escape onto the invisible platform.

10 The test can be adapted to assess several aspects of spatial memory: a) acquisition of a cued task, where the animal's ability to link one visual cue directly with the escape platform depends on cortical function (i.e. a ball is suspended over the escape platform and the animal
15 learns to follow this cue to find the platform); b) acquisition of a spatial task, where the animal's ability to learn the location of a submerged escape platform based on a combination of distant visual cues is dependent upon hippocampal function (i.e. the animal learns to
20 triangulate its position in the tank by visually aligning the paper-tower dispenser with the door and ceiling lamp); c) retention of a successfully acquired spatial task, which is predominantly dependant on cortical function (i.e. the animal must remember the spatial location of the
25 platform over several weeks); d) a hippocampus-dependant reversal task where the animals must reacquire a new spatial platform location (i.e. the platform is moved to a new location between swim trials and the animal must abandon its previous search strategy and acquire a new
30 one).

These different modifications of the Morris watermaze procedure can be applied in sequence to the same set of experimental animals and allow for a thorough
35 characterization of their spatial memory performance and its decline with normal ageing. Moreover, such a series

of sequential memory tests sheds some light on the functional integrity of the specific brain systems involved in the acquisition and retention of spatial memory (e.g. rats with cholinergic lesions of the hippocampus may remember a platform location acquired weeks before, but persevere over the old platform location after the platform is moved).

Effects of Chronic GPI-1046 Administration

on Spatial Learning and Memory in Aged Rodents

This Example shows the effects of chronic treatment with the systemically available FKBP-ligand GPI-1046 on spatial learning and memory in aged rodents.

The procedure involved using three-month old (young) and 18-19 month old male C57BL/6N-Nia (aged) mice which habituated to the well known and conventional Morris watermaze during a 4 trials/day, 3-4 day visible platform training phase. Subsequent spatial acquisition testing was conducting as follows: All mice were given 4 trials/day (block), for 5 days. Maximum swim time was 90 seconds. Aged mice were allocated to an "aged impaired" group if their performance during blocks 4 or 5 of the acquisition phase was >1 S.D. above the mean of "young" mice, and to an "aged non-impaired" group if their performance was < 0.5 S.D. above the mean of "young" mice. Aged groups were then split into statistically similar "GPI-1046" and "vehicle" groups.

Daily treatment with 10mg/kg GPI-1046 was initiated 3 days after the end of acquisition training, and continued through retention testing. Retention testing began after 3 weeks of dosing using the same methods as the acquisition phase. Swim Distances (cm) were analyzed in a 7 X 5 ANOVA including Groups and Blocks (1-5) as factors in the analysis, treating Blocks as a repeated measure.

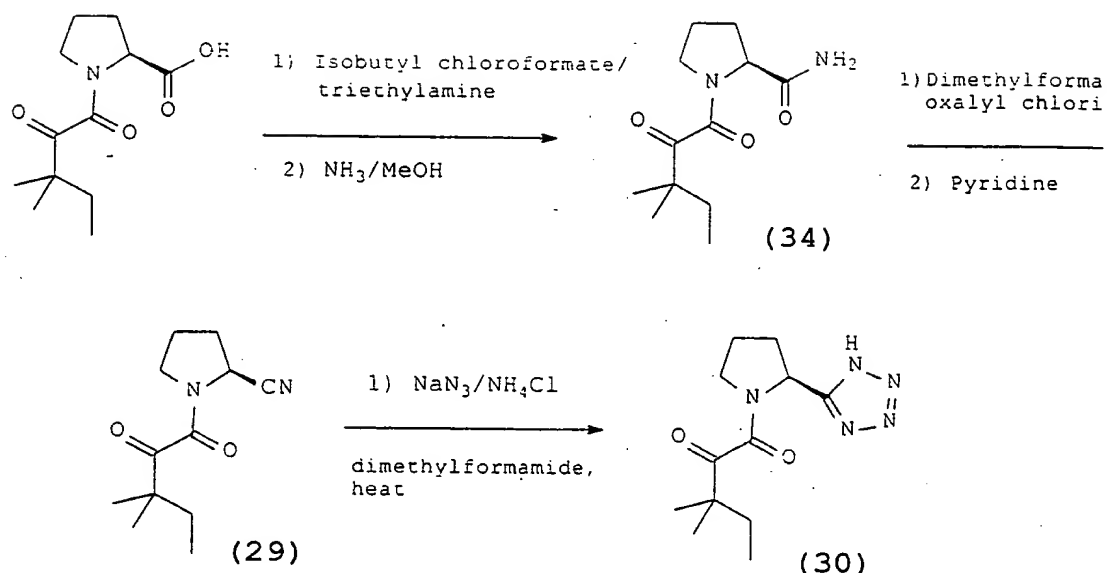
The results showed that planned contrasts revealed

that there were significant differences between the "young", and "aged impaired-vehicle and GPI-1046" treated groups at the end of the acquisition phase, $F_{1,58} = 26.75$, $P=0.0001$, and $F_{1,58} = 17.70$, $P=0.0001$ respectively. While there were no significant differences between the two "aged impaired" groups, $F_{1,58} = 0.67$, $P = 0.42$. During retention testing, however, "aged impaired-vehicle" treated animals performed significantly poorer than "aged impaired - GPI-1046", and "young" animals, $F_{1,69} = 8.11$, $P = 0.006$, and $F_{1,69} = 25.45$, $P = 0.0001$ respectively. There was no longer any statistically significant difference between the "young" and "aged impaired" - GPI-1046" treated groups during the retention phase, $F_{1,69} = 3.09$, $P = 0.08$. In summary, systemic treatment with GPI-1046 significantly enhanced spatial memory performance of mice with age-related spatial memory impairments.

EXAMPLES

The inventive compounds may be prepared by a variety of synthetic sequences that utilize established chemical transformations. A pathway to the compounds of Examples 1 through 4 is described in Scheme I. N-glyoxylproline derivatives may be prepared by reacting L-proline methyl ester with methyl oxalyl chloride as shown in Scheme I. The resulting oxamates may be reacted with a variety of carbon nucleophiles to obtain compounds used in the present invention.

Scheme I



EXAMPLE 1

5 Synthesis of (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate. (Compound 137)

a. Synthesis of (2S)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate.

A solution of L-proline methyl ester hydrochloride (3.08 g; 18.60 mmol) in dry methylene chloride was cooled to 0°C and treated with triethylamine (3.92 g; 38.74 mmol; 2.1 eq). After stirring the formed slurry under a nitrogen atmosphere for 15 min, a solution of methyl oxalyl chloride (3.20 g; 26.12 mmol) in methylene chloride (45 mL) was added dropwise. The resulting mixture was stirred at 0°C for 1.5 hr. After filtering to remove solids, the organic phase was washed with water, dried over MgSO_4 and concentrated. The crude residue was purified on a silica gel column, eluting with 50% ethyl acetate in hexane, to obtain 3.52 g (88%) of the product as a reddish oil. Mixture of cis-trans amide rotamers; data for trans rotamer given. ^1H NMR (CDCl_3): δ 1.93 (dm, 2H); 2.17 (m, 2H); 3.62 (m, 2H); 3.71 (s, 3H); 3.79, 3.84 (s, 3H total); 4.86 (dd, 1H, $J = 8.4, 3.3$).

25 b. Synthesis of methyl (2S)-1-(1,2-dioxo-3,3-

dimethylpentyl)-2-pyrrolidinecarboxylate.

A solution of methyl (2S)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate (2.35 g; 10.90 mmol) in 30 mL of tetrahydrofuran (THF) was cooled to -78°C and treated with 14.2 mL of a 1.0 M solution of 1,1-dimethylpropylmagnesium chloride in THF. After stirring the resulting homogeneous mixture at -78°C for three hours, the mixture was poured into saturated ammonium chloride (100 mL) and extracted into ethyl acetate. The organic phase was washed with water, dried, and concentrated, and the crude material obtained upon removal of the solvent was purified on a silica gel column, eluting with 25% ethyl acetate in hexane, to obtain 2.10 g (75%) of the oxamate as a colorless oil. ¹H NMR (CDCl₃): δ 0.88 (t, 3H); 1.22, 1.26 (s, 3H each); 1.75 (dm, 2H); 1.87-2.10 (m, 3H); 2.23 (m, 1H); 3.54 (m, 2H); 3.76 (s, 3H); 4.52 (dm, 1H, J = 8.4, 3.4).

c. Synthesis of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid (Compound 137).

A mixture of methyl (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylate (2.10 g; 8.23 mmol), 1 N LiOH (15 mL), and methanol (50 mL) was stirred at 0°C for 30 min and at room temperature overnight. The mixture was acidified to pH 1 with 1 N HCl, diluted with water, and extracted into 100 mL of methylene chloride. The organic extract was washed with brine and concentrated to deliver 1.73 g (87%) of snow-white solid which did not require further purification. ¹H NMR (CDCl₃): δ 0.87 (t, 3H); 1.22, 1.25 (s, 3H each); 1.77 (dm, 2H); 2.02 (m, 2H); 2.17 (m, 1H); 2.25 (m, 1H); 3.53 (dd, 2H, J = 10.4, 7.3); 4.55 (dd, 1H, J = 8.6, 4.1).

Inventive compounds containing bridged rings may be synthesized using the above synthetic schemes by substituting the substrates containing the N-heterocyclic

ring structures with comparable substrates containing bridged ring structures.

EXAMPLE 2

5 Synthesis of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxamide (Compound 34).

This example was prepared according to the process of Scheme II as follows.

Isobutyl chloroformate (20 mmol, 2.7 mL) was added to
10 a solution containing (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid (4.89 g, 20 mmol) (from Example 1) in 50 mL methylene chloride at -10°C with stirring. After 5 minutes, ammonia was added dropwise (20 mmol, 10 mL of 2 M ethyl alcohol solution).
15 The reaction was warmed up to room temperature after stirring at -10°C for 30 minutes. The mixture was diluted with water, and extracted into 200 mL methylene chloride. The organic extract was concentrated and further purified by silica gel to give 4.0 g of product as a white solid
20 (81.8% yield). ¹H NMR (CDCl₃): δ 0.91 (t, 3H, J= 7.5); 1.28 (s, 6H, each); 1.63-1.84 (m, 2H); 1.95-2.22 (m, 3H); 2.46 (m, 1H); 3.55-3.67 (m, 2H); 4.67 (t, 1H, J= 7.8); 5.51-5.53 (br, 1H, NH); 6.80 (br, 1H, NH).

EXAMPLE 3

25 Synthesis of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarbonitrile (Compound 29).

This example was prepared according to the process of Scheme III as follows.

30 To a solution of 0.465 mL DMF (6 mmol) in 10 mL acetonitrile at 0°C was added 0.48 mL (5.5 mmol) of oxalyl chloride. A white precipitate formed immediately and was accompanied by gas evolution. When complete, a solution of 1.2 g (5 mmol) of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxamide (from Example 2)
35

in 2.5 mL acetonitrile was added. When the mixture became homogeneous, 0.9 mL (11 mmol) pyridine was added. After 5 min., the mixture was diluted into water and extracted by 200 mL ethyl acetate. The organic layer was concentrated and further purified by silica gel to give 0.8 g product as a white solid (72% yield). ¹H NMR (CDCl₃): δ 0.87 (t, 3H, J= 7.5); 1.22 (s, 3H); 1.24 (s, 3H); 1.80 (m, 2H); 2.03-2.23 (m, 4H); 3.55 (m, 2H); 4.73 (m, 1H).

EXAMPLE 4

Synthesis of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinetetrazole (Compound 30).

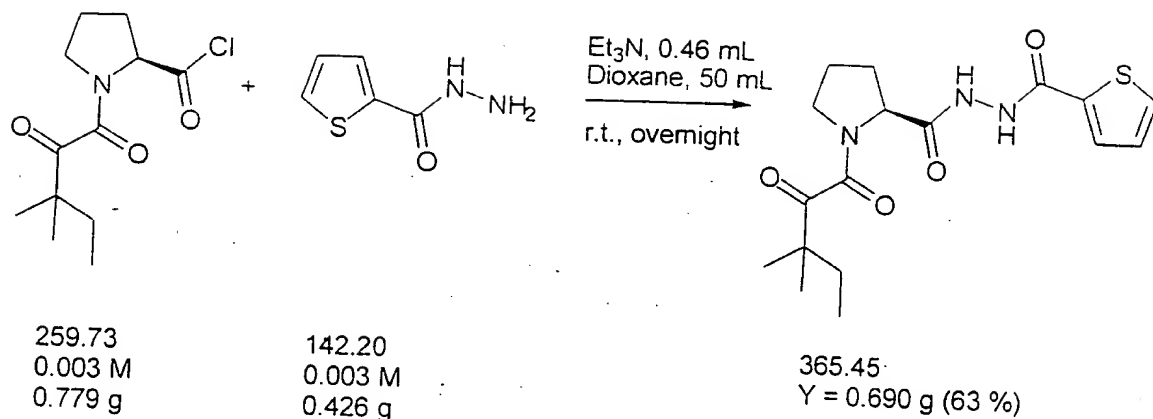
This example was prepared according to the process of Scheme IV as follows.

A mixture of (2S)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarbonitrile (222 mg, 1 mmol) (from Example 3), NaN₃ (81 mg, 1.3 mmol) and NH₄Cl (70 mg, 1.3 mmol) in 3 mL DMF was stirred at 130°C for 16 hours. The mixture was concentrated and purified by silica gel to afford 200 mg product as white solid (75.5% yield). ¹H NMR (CDCl₃): δ 0.88 (t, 3H, J= 7.5); 1.22 (s, 6H); 1.68 (m, 2H); 2.05-2.36 (m, 3H); 2.85 (m, 1H); 3.54 (m, 1H); 3.75 (m, 1H); 5.40 (m, 1H).

Example 5

[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-N-(2-thienylcarbonylamino)-formamide; molecular formula: C₁₇H₂₃N₃O₄S; molecular weight: 365.45 (Compound 140).

This example was prepared according to the process of Scheme V as follows.

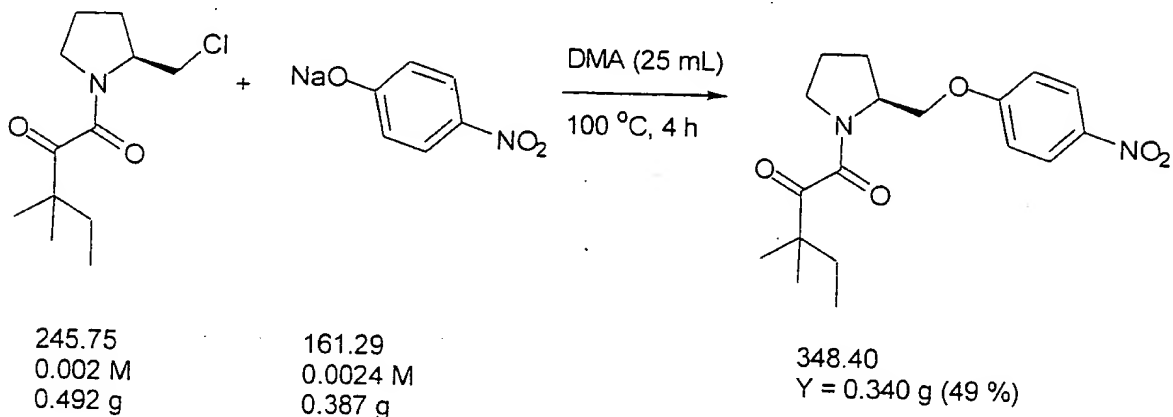


To a solution of thiophene carbonyl hydrazide (0.426 g, 3 mmol) and triethylamine (0.460 mL, 3.3 mmol) in dioxane (40 mL) was added dropwise a solution of acid chloride (0.779 g, 3 mmol) in dioxane (10 mL) under stirring at room temperature within 5-7 min (immediate precipitation of triethylamine hydrochloride was observed as soon as first few drops were added). After addition is completed, the whole was stirred overnight at room temperature. The suspension formed was poured onto ice-water (100 g), and stirred for 15 min. Dichloromethane (50 mL) was added, and the reaction product was extracted (separating funnel) into organic layer. It was separated, dried over Na_2SO_4 (anhyd), filtered, and organic solvents were evaporated in vacuo. The oily solid obtained (0.690 g, 63%) was subjected to the column chromatography (silica gel, eluent - EtOAc:hexanes, 2:1). Fractions with R_f ca. 0.35 were collected. Evaporation of solvents gave 0.130 g of white microcrystals with m.p. 72-74 °C. ^1H NMR (CDCl_3 , 400 MHz): d 9.51 (br s, 1H); 9.31 (br s, 1H); 7.66-7.63 (m, 1H); 7.50-7.46 (m, 1H); 7.02-6.98 (m, 1H); 4.68-4.63 (m, 1H); 3.53-3.48 (m, 2H); 2.33-1.60 (m, 6H); 1.26 (s, 3H); 1.21 (s, 3H); 0.86 (t, $J=7.5$, 3H) Calcd for $\text{C}_{17}\text{H}_{23}\text{N}_3\text{O}_4\text{S}$: C, 55.87; H, 6.34; N, 11.50; S, 8.77. Found: C, 55.79; H, 6.57; N, 11.20; S, 8.52.

Example 6

3,3-Dimethyl-1-(2-[(4-nitrophenoxy)methyl]pyrrolidinyl)pentane-1,2-dione; molecular formula: $C_{18}H_{24}N_2O_5$; molecular weight: 348.40 (Compound 141).

5 This example was prepared according to the process of Scheme VI as follows.



10 A solution of sodium 4-nitrophenolate (0.387 g, 2.4 mmol; prepared from NaOH and 4-nitrophenol in refluxing ethanol) and chloride (0.492 g, 2 mmol) in DMA (25 mL) were heated and stirred for 4 h. The mixture was poured onto ice-water (100 g), and organic products were extracted by

15 dichloromethane (2x50 mL). Organic layer was separated, excessively washed with water (6x30 mL), separated, dried over Na_2SO_4 (anhyd), filtered, and organic solvents were evaporated in vacuo. The brown oil obtained (0.340 g, 49%) was twice subjected to the column chromatography

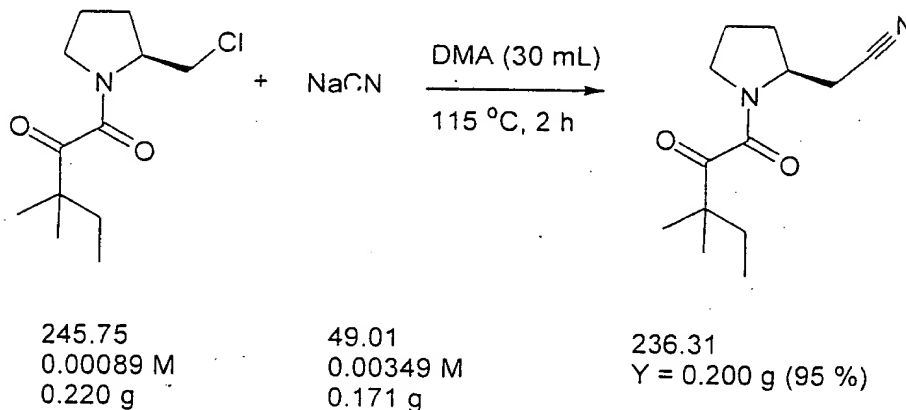
20 (silica gel, eluent - EtOAc:hexanes, 1:1). Fractions with R_f ca. 0.45 were collected. Evaporation of solvents gave 0.145 g of yellow oil. ^1H NMR (CDCl_3 , 400 MHz): d 8.19 (d, $J=7.1$, 2H); 7.03-6.92 (m, 2H); 4.51-4.42 (m, 1H); 4.35-4.22 (m, 2H); 3.50-3.42 (m, 2H); 2.18-1.64 (m, 6H); 1.20 (s, 3H); 1.19 (s, 3H); 0.86 (t, $J=7.5$, 3H). Calcd for

25 $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 61.91; H, 7.02; N, 7.80.

Example 7

2-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]ethanenitrile; molecular formula: $C_{13}H_{20}N_2O_2$; molecular weight: 236.31 (Compound 28).

5 This example was prepared according to the process of Scheme VII as follows.



10 A mixture of chloride (0.220 g, 0.89 mmol) and sodium cyanide (0.171 g, 3.49 mmol) in DMA (30 mL) was stirred upon heating at 115 °C. After cooling to a room temperature, water (50 mL) was added to the mixture, and the whole was stirred for 30 min. Diethyl ether (50 mL) was added, and the organic products were extracted
15 (separating funnel) into organic layer, which was separated and dried over $MgSO_4$ (unhyd). Evaporation of solvents gave light yellow oil, which still contained DMA (NMR). Extraction was repeated using mixture of dichloromethane (50 mL) and water (30 mL). Separation of
20 organic layer, drying over Na_2SO_4 (anhyd), evaporation of the solvent, and pumping of the product on the vacuum line (5 mm Hg, 2 days) gave white solid, which was subjected to the column chromatography (silica gel, eluent - EtOAc:hexanes, 1:2). Fractions with R_f ca. 0.65 were
25 collected. Evaporation of solvents gave 0.115 g of white waxy solid with m.p. 91-93 °C. 1H NMR ($CDCl_3$, 400 MHz): d 4.21 (dd, $J=3.6, 11.6$ Hz, 1H); 3.77-3.63 (m, 2H); 3.58-

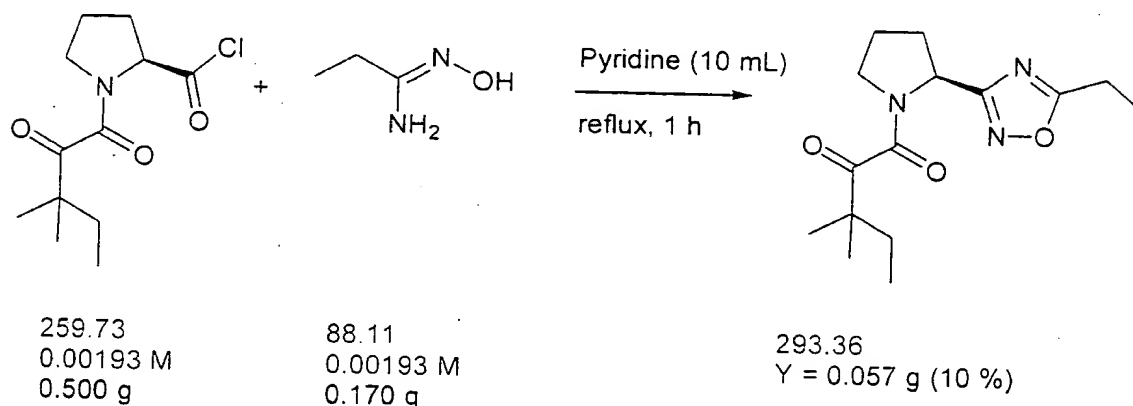
3.41 (m, 2H); 2.13-2.03 (m, 2H); 1.95-1.78 (m, 1H); 1.74-1.66 (m, 1H); 1.53-1.41 (m, 2H); 1.46 and 1.14 (two s, 3+3 H); 0.90 (t, J=7.5 Hz, 3H). Calcd for $C_{13}H_{23}N_2O_2$: C, 66.07; H, 8.53; N, 11.85. Found: C, 66.24; H, 8.51; N, 11.93.

5

Example 8

1-[2-(3-Ethyl(1,2,4-oxadiazol-5-yl))pyrrolidiny]l]-3,3-dimethylpentane-1,2-dione; molecular formula: $C_{15}H_{23}N_3O_3$; molecular weight: 293.36 (Compound 142).

10 This example was prepared according to the process of Scheme VIII as follows.



15 To a stirred suspension of amidoxime (0.170 g, 1.93 mmol) in dry pyridine was added an acid chloride (0.500 g, 1.93 mmol) under nitrogen at room temperature. The whole was brought to reflux; stirring and refluxing was continued for 1 h. The brownish-colored solution was cooled down to room temperature, diluted with water (30 mL), and

20 extracted with EtOAc (3x30 mL). Combined organic layers were washed with water (50 mL), HCl (1N in water, 150 mL), separated, and dried over $MgSO_4$. Filtration and evaporation of the solvents in vacuo gave yellowish oil. It was

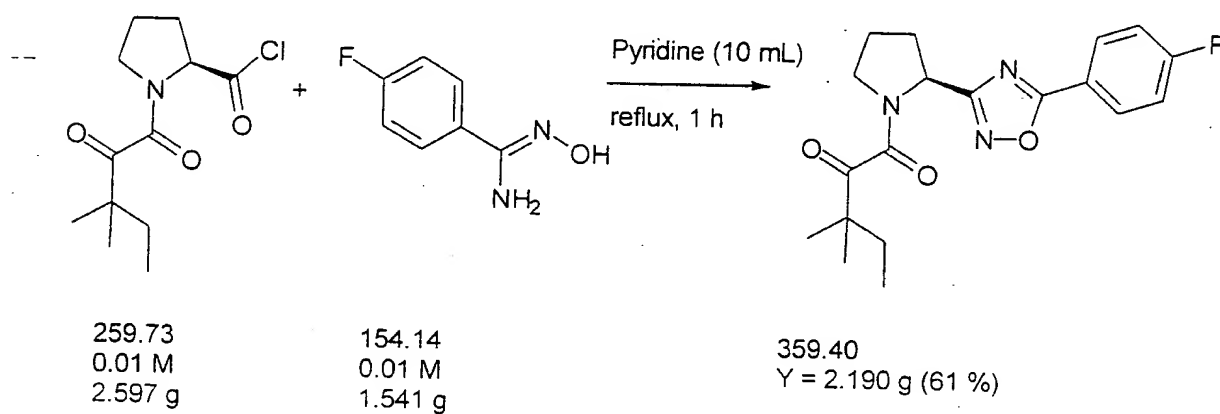
25 purified by column chromatography (silica gel, eluent - EtOAc:hexanes, 1:1) to give a clear oil (0.057 g, 10%). 1H NMR ($CDCl_3$, 400 MHz): δ 5.31 (d, 1H); 3.62-3.65 (m, 2H); 2.71-2.76 (m, 2H); 2.06-2.15 (m, 4H); 1.64-1.85 (m, 2H); 1.32 (t, J = 7.2 Hz, 3H); 1.24 (s, 3H); 1.22 (s, 3 H); 0.88

(t, J=7.5 Hz, 3H). Calcd for $C_{15}H_{23}N_3O_3$: C: 61.41; H: 7.90; N: 14.32. Found: C: 61.22; H: 7.87; N: 13.76.

Example 9

5 1-(2-[3-(4-Fluorophenyl)(1,2,4-oxadiazol-5-yl)]pyrrolidinyl)-3,3-dimethylpentane-1,2-dione;
molecular formula: $C_{19}H_{22}FN_3O_3$; molecular weight: 359.40
(Compound 143).

This example was prepared according to the process of
10 Scheme IX as follows.



The procedure is identical to that used in the previous
case. Crude product was isolated as a yellow oil (2.190 g,
15 61%) and was purified by column chromatography (silica
gel; eluent - EtOAc:hexanes 1:1). Fractions with R_f ca.
0.65 were collected. Evaporation of solvents gave 1.150 g
of light-yellow oil. 1H NMR ($CDCl_3$, 400 MHz): δ 8.08-8.04
(m, 2H); 7.19-7.14 (m, 2H); 5.56-5.51 and 5.42-5.37 (2m -
2 rotamers, 1H); 3.91-3.84 and 3.75-3.65 (2m - 2 rotamers,
20 2H); 2.50-2.05 (m, 4H); 1.85-1.60 (m, 2H); 1.27, 1.24,
1.19, and 1.09 (4s - 2 rotamers, 3H); 0.88 (t, J=7.5, 3H).
Calcd for $C_{19}H_{22}FN_3O_3$: C: 63.50; H: 6.17; N: 11.69. Found: C:
63.58; H: 6.16; N: 11.70.

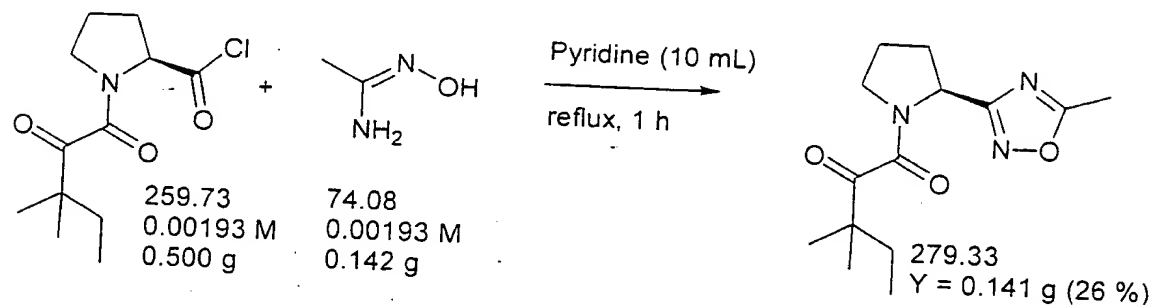
25

Example 10

3,3-Dimethyl-1-[2-(3-methyl(1,2,4-oxadiazol-5-yl))pyrrolidinyl]pentane-1,2-dione; molecular formula:

$C_{14}H_{21}N_3O_3$; molecular weight: 279.33 (Compound 144).

This example was prepared according to the process of Scheme X as follows.

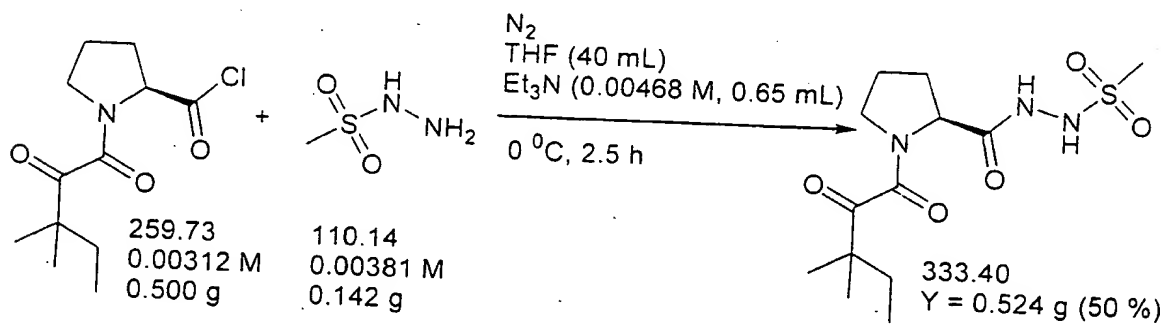


10 The procedure is identical to that used in the previous case. Crude product was isolated as a yellow oil and was purified by column chromatography (silica gel; eluent - EtOAc:hexanes 1:1). Fractions with R_f ca. 0.75 were collected. Evaporation of solvents gave 0.141 g of light-yellow oil. 1H NMR ($CDCl_3$, 400 MHz): δ ; 5.35 (m, 1H); 3.64 (m, 2H); 2.38 (s, 3H); 2.07-2.10 (m, 4H); 1.68-1.76 (m, 2H); 1.21 (s, 3H); 1.19 (s, 3H); 0.86 (t, $J=7.5$ Hz, 3H). Calcd for $C_{14}H_{21}N_3O_3$: C: 60.20; H: 7.58; N: 15.04; found: C: 60.05; H: 7.72; N: 14.91.

Example 11

20 [1-(3,3-Dimethyl-2-oxopent-1-en-1-yl)pyrrolidin-2-yl]-N-[(methylsulfonyl)amino]-formamide; molecular formula: $C_{13}H_{23}N_3O_5S$; molecular weight: 333.40 (Compound 145).

This example was prepared according to the process of Scheme XI as follows.



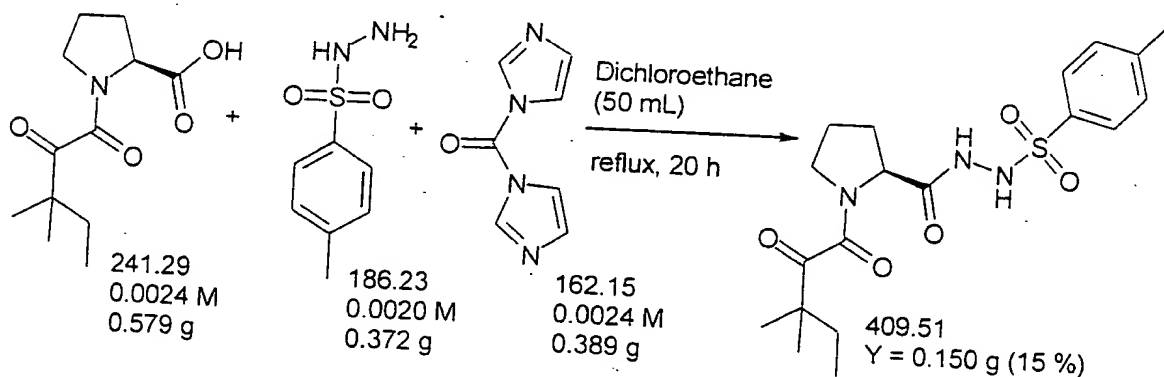
Sulfonyl hydrazide (0.142 g, 3.81 mmol) and triethylamine

(0.65 mL, 4.68 mmol) were dissolved in dry THF (30 mL) under stirring at 0 °C. A solution of acid chloride (0.500 g, 3.12 mmol) in dry THF (10 mL) was added dropwise within 10 min, and stirring was continued for another 2 h 20 min at 0 °C. A mixture was diluted with dichloromethane (50 mL), washed with water (2x50 mL), and aqueous NaHCO₃ (10% w/w, 50 mL). Organic layer was separated, dried over MgSO₄ (anhyd), filtered. Removal of the solvents *in vacuo* gave crude product as a pink oil, which was purified by column chromatography (silica gel, eluent - EtOAc:hexanes 3:1). Fractions with R_f ca. 0.50 were collected; evaporation of the solvents gave 0.524 g of puffy white solid. ¹H NMR (CDCl₃, 400 MHz): δ 8.90 (br s, 1H); 6.80 (br s, 1H); 4.50 (m, 1H); 3.52 (m, 2H); 3.01 (s, 3H); 2.07-2.10 (m, 4H); 1.68-1.76 (m, 2H); 1.21 (s, 3H); 1.19 (s, 3H); 0.86 (t, J=7.5 Hz, 3H). Calcd for C₁₃H₂₃N₃O₅S: C: 46.83; H: 6.95; N: 12.60, S: 9.62. Found: C: 47.16; H: 7.26; N: 12.29; S: 9.39.

Example 12

[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-N-[[4-methylphenyl)sulfonyl]-amino}formamide; molecular formula: C₁₉H₂₇N₃O₅S; molecular weight: 409.51 (Compound 146).

This example was prepared according to the process of Scheme XII as follows.

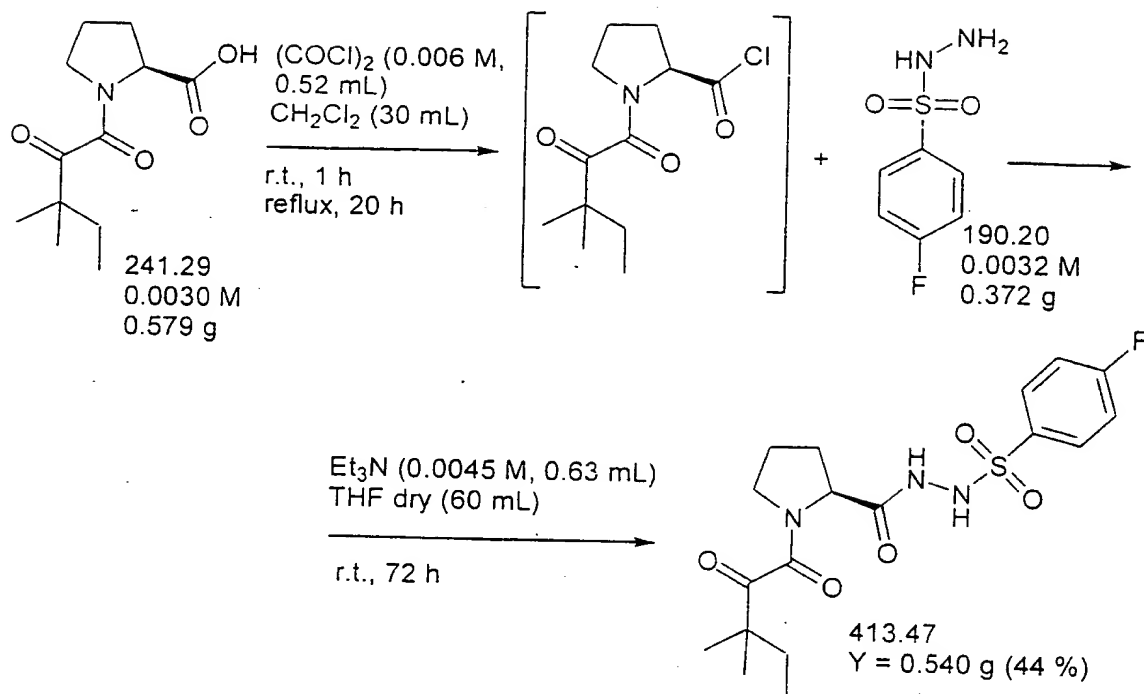


To a stirred solution of the acid (0.579 g, 2.4 mmol) and sulfonyl hydrazide (0.372 g, 2.0 mmol) in dry 1,2-dichloroethane was added carbonyl-bis-imidazole (0.389 g, 2.4 mmol) in portions over 10 min period at room temperature. Stirring at room temperature was continued until the gas evolution was ceased. The mixture was then refluxed for 20 h, and solvent was evaporated *in vacuo*. The oil formed was dissolved in chloroform (50 mL), and washed with Na₂CO₃ (sat., 20 mL) and water (20 mL). Organic layer was separated, dried over Na₂SO₄ (anhyd), filtered, and the solvent was evaporated *in vacuo* to give crude product in the form of heavy oil. It was purified by column chromatography (silica gel, eluent: EtOAc:hexanes, 1:1). Fractions with R_f ca. 0.30 were collected; evaporation of the solvents gave 0.150 g of white solid, which was triturated with a mixture of ether:hexanes (1:5 v/v, 15 mL). Filtration of the suspension afforded 0.100 g of analytically pure product as white microcrystals with m.p. 59-61 °C. ¹H NMR (CDCl₃, 400 MHz): δ 9.08 (br s, 1H); 7.78 (d, J=7.5, 2H); 7.29 (d, J=7.5, 2H); 4.44-4.39 (m, 1H); 3.39-3.35 (m, 2H); 2.42 (s, 3H); 1.93-1.68 (m, 6H); 1.24-1.13 (m, 6H); 0.86 (t, J=5.3, 3H). Calcd for C₁₉H₂₇N₃O₅S: C, 55.73; H, 6.65; N, 10.26; S, 7.83. Found: C, 55.49; H, 6.63; N, 10.14; S, 7.85.

Example 13

[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-N-([(4-fluorophenyl)sulfonyl]-amino)formamide; molecular formula C₁₃H₂₄FN₃O₅S; molecular weight 413.47 (Compound 147).

This example was prepared according to the process of Scheme XIII as follows.



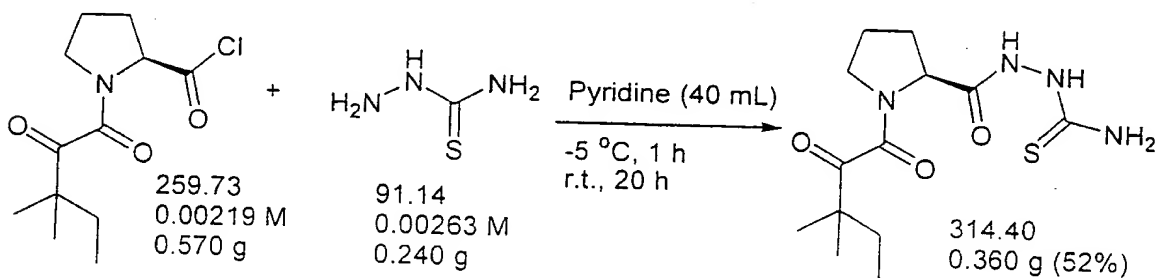
A solution of acid (0.724 g, 3.0 mmol) and oxalyl chloride (0.52 mL, 6.0 mmol) in dichloromethane (30 mL) was stirred at room temperature for 1 h. Within this time the gas evolution became steady, then the mixture was brought to reflux, and refluxing and stirring were continued overnight. The solvent was removed *in vacuo*, resulting yellow oil was dissolved in dry THF (10 mL) and was added dropwise to a stirred solution of hydrazide (0.372 g, 3.2 mmol) and triethylamine (0.63 mL, 4.5 mmol) in dry THF (50 mL) at room temperature. The formed white suspension was stirred for 72 h, then transferred into separating funnel. Dichloromethane (30 mL) and HCl (1N, 30 mL) were added, and the whole was shaken for 5 min. Organic layer was separated, washed with aq. Na_2CO_3 (30% w/w, 75 mL), then with water (50 mL), separated, dried over Na_2SO_4 (anhyd), filtered, and the solvent was evaporated *in vacuo* to give 0.540 g of heavy oil. Crude product was purified by column chromatography (silica gel, eluent: EtOAc:hexanes, 2:1). Fractions with R_f ca. 0.65 were collected; evaporation of the solvents gave 0.100 g of colorless oil, which solidified upon standing; m.p. 57–60 °C. ^1H NMR (CDCl_3 , 400

MHz): d 9.22 (br s, 1H); 7.98-7.92 (m, 2H); 7.70 (br s, 1H); 7.22-7.16 (m, 2H); 4.42-4.38 (m, 1H); 3.42-3.43 (m, 2H); 2.02-1.62 (m, 6H); 1.19 and 1.18 (2 s, 6H); 0.86 (t, J=7.5 Hz, 3H). Calcd for $C_{18}H_{24}FN_3O_5S \cdot 0.17 H_2O$: C, 51.90; H, 5.89; N, 10.09; S, 7.70. Found: C, 52.29; H, 6.17; N, 9.62; S, 7.31.

Example 14

(2S)-3,3-Dimethyl-1-[2-(5-sulfanyl(4H-1,2,4-triazol-3-yl))pyrrolidinyl]-pentane-1,2-dione; molecular formula: $C_{13}H_{20}N_4O_2S$; molecular weight: 296.393 (Compound 148).

This example was prepared according to the process of Scheme XIV as follows.



A solution of acid (0.598 g, 2.48 mmol) and oxalyl chloride (0.22 mL, 2.48 mmol) in dichloromethane (30 mL) was stirred at room temperature for 1 h. Within this time the gas evolution became steady, then the mixture was brought to reflux, and refluxing and stirring were continued overnight. The solvent was removed *in vacuo*. Resulting oil was treated with a solution of thiosemicarbazide (0.294 g, 3.22 mmol) and triethylamine (0.45 mL, 3.22 mmol) in DMA (15 mL) upon stirring at room temperature for 20 h. A solution obtained was poured into water (150 mL) and extracted with dichloromethane (3x30 mL). Combined organic extracts were dried over Na_2SO_4 (anhyd), and the solvent was evaporated *in vacuo*. The crude intermediate was kept *in vacuo* for 6 h, and then dissolved in NaOH (1N, 10 mL). The solution was stirred

and heated at 60 °C for 3 h, and then at room temperature -
for 2 h. It was then acidified with HCl (1N, to pH ca. 1),
and extracted with dichloromethane (2x30 mL). Combined
organic layers were washed with water (2x30 mL),
5 separated, dried over Na₂SO₄ (anhyd), and the solvent was
evaporated in vacuo to give 0.060 g of amber oil. It was
kept in vacuo for 60 h to form solid, which was purified
by column chromatography (silica gel, eluent:
EtOAc:hexanes, 2:1). Fractions with R_f ca. 0.30 were
10 collected; evaporation of solvents gave 0.025 g of white
foamy solid. ¹H NMR (CDCl₃, 400 MHz): d 12.2 (br s, 1H);
11.6 (br s, 1H); 5.19 (dd, J=3.6, 5.1 Hz, 1H); 3.58-3.45
(m, 2H); 2.58-2.50 (m, 1H); 2.29-2.11 (m, 2H); 2.07-2.00
(m, 1H); 1.72 (q, J=1.4, 6.2, 2H); 1.22-1.17 (m, 6H); 0.87
15 (t, J=4.4 Hz, 3H). ¹³C NMR (CDCl₃, 125 MHz) d 206.2, 166.6,
166.5, 151.9, 52.3, 47.7, 47.0, 32.3, 27.8, 24.9, 23.6,
23.4, 8.9. Calcd for C₁₃H₂₀N₄O₂S: C, 52.68; H, 6.80; N,
18.90; S, 10.82. Found: C, 52.70; H, 6.81; N, 18.66; S,
10.94.

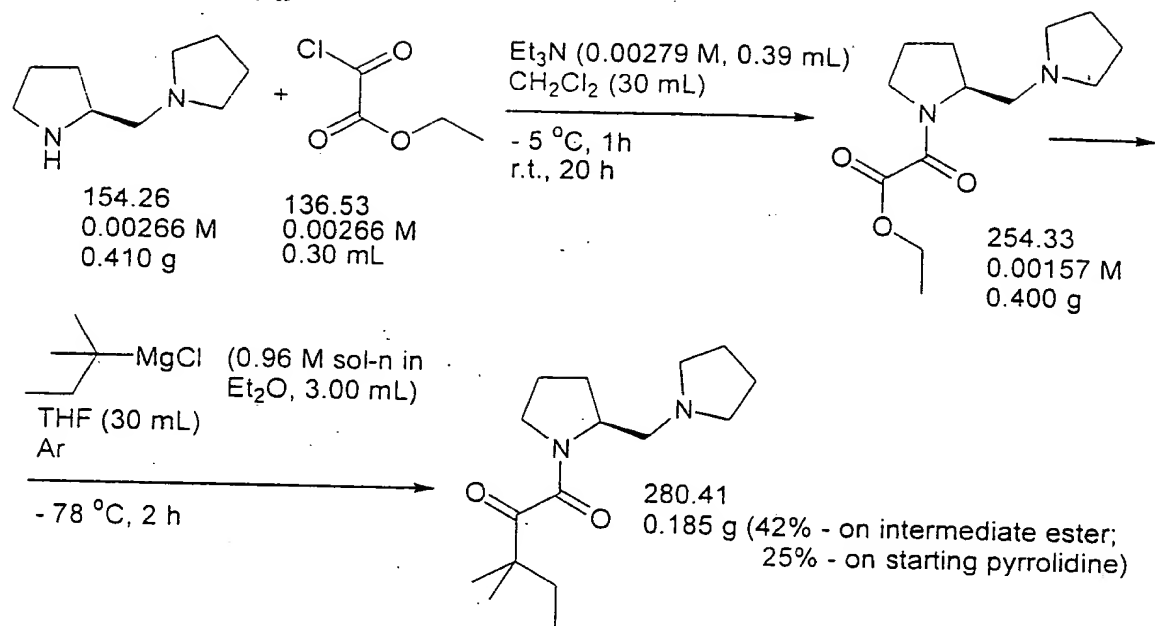
20

Example 15

(2S)-3,3-Dimethyl-1-[2-(pyrrolidinylmethyl)pyrrolidinyl]
pentane-1,2-dione; molecular formula: C₁₆H₂₈N₂O₂; molecular
weight: 280.409 (Compound 149).

25

This example was prepared according to the process of
Scheme XV as follows.



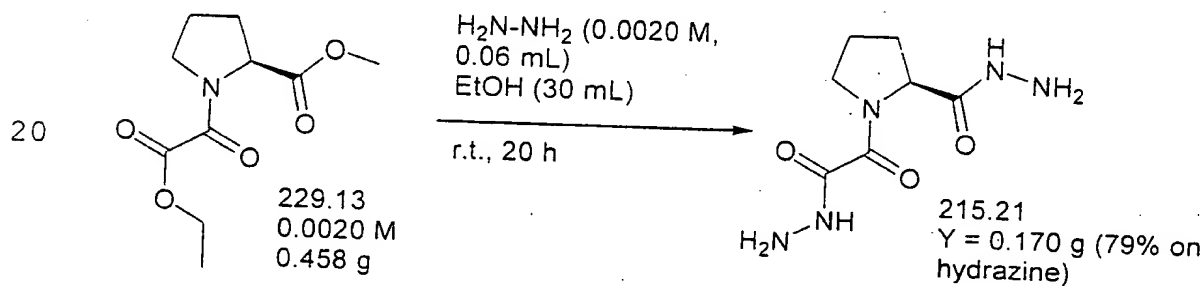
A solution of ethyl chlorooxoacetate (0.30 mL, 2.66 mmol) in dichloromethane (10 mL) was added dropwise within 5 min to a stirred and chilled solution of 2-(pyrrolidin-1-yl)pyrrolidine (0.410 g, 2.66 mmol) and triethylamine (0.39 mL, 2.79 mmol) in dichloromethane (20 mL). After addition was completed, the stirring was continued at -5 °C for another 50-55 min, and at room temperature for 20 h. Water (50 mL) and dichloromethane (30 mL) were added, and the mixture was transferred into separating funnel. After extraction, organic layer was separated and dried over Na₂SO₄ (anhyd). Evaporation of the solvent *in vacuo* gave 0.400 g of heavy yellow oil. It was kept *in vacuo* for 20 h, then dissolved in dry THF (30 mL) and chilled to -78 °C upon stirring. An ethereal solution of Grignard reagent (0.96 M, 3.00 mL) was added dropwise to the latter solution within 15-20 min, and the whole was stirred for another 1 h 45 min. A mixture was quenched by addition of NH₄Cl (sat., 20 mL) and water (50 mL), stirred for 10 min, and extracted with dichloromethane (2x50 mL). Combined organic layers were washed with water (50 mL) and dried over Na₂SO₄ (anhyd). Evaporation of the solvent *in*

vacuo gave 0.370 g of yellow oil. Crude product was purified by column chromatography (silica gel, eluent: CHCl_3 :MeOH, 10:1). Fractions with R_f ca. 0.10 (in EtOAc:hexanes, 2:1) were collected; evaporation of solvents gave 0.185 g of heavy yellow oil. ^1H NMR (CDCl_3 , 400 MHz): δ 4.29-4.22 and 3.99-3.92 (two rotamers, m, 1H); 3.61-3.52 and 3.47-3.39 (two rotamers, m, 2H); 2.73-2.43 (complex m, 6H); 2.14-1.68 (complex m, 10H); 1.23-1.20 (two rotamers, set of singlets, 6H); 0.87 (t, $J=7.5$ Hz, 3H). Calcd for $\text{C}_{16}\text{H}_{28}\text{N}_2\text{O}_2$: C, 68.53; H, 10.06; N, 9.99. Found: C, 68.78; H, 9.87; N, 9.77.

Example 16

(2S)-N-[(Aminothioxomethyl)amino][1-(3,3-dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]formamide; molecular formula: $\text{C}_{13}\text{H}_{22}\text{N}_4\text{SO}_3$; molecular weight: 314.41 (Compound 150).

This example was prepared according to the process of Scheme XVI as follows.



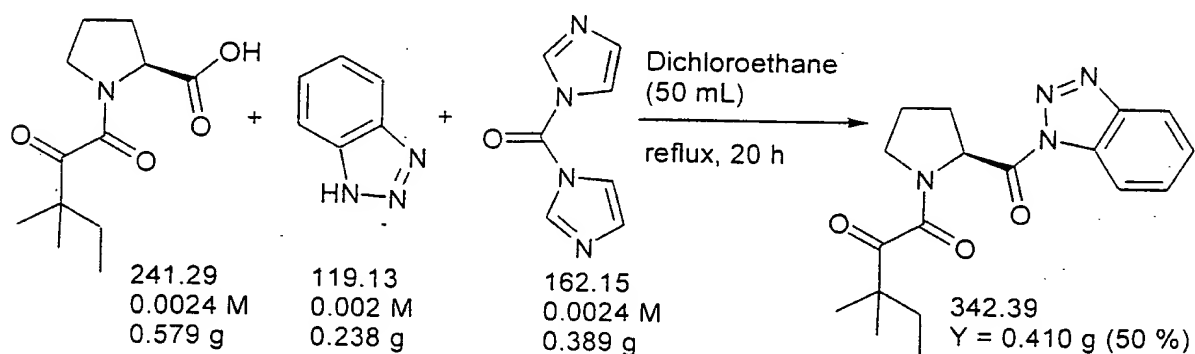
A solution of acid chloride (0.570 g, 2.19 mmol) in dry pyridine (10 mL) was added to a stirred and chilled suspension of thiosemicarbazide (0.240 g, 2.63 mmol) in dry pyridine (30 mL). The mixture was allowed to warm up to room temperature, and stirring was continued for 20 h. Solvent was evaporated in vacuo, hot water (50 mL) was added to the residue, and the whole was stirred for 5 min

at room temperature. NH_4Cl (sat., 20 mL) was added to the mixture, followed by extraction with dichloromethane (2x50 mL). After separation, combined organic layers were dried over Na_2SO_4 (anhyd). Evaporation of the solvent *in vacuo* gave 0.360 g of oily yellowish solid. Crude product was purified by column chromatography (silica gel, eluent: EtOAc:hexanes, 3:1 to 5:1). Fractions with R_f ca. 0.20 were collected; evaporation of solvents gave 0.077 g of white puffy solid. ^1H NMR (CDCl_3 , 400 MHz): d 9.60 (br s, 1H); 8.40 (br s, 1H); 7.20-7.30 (m, 2H); 4.53-4.56 (m, 1H); 3.54-3.55 (m, 2H); 2.18-2.19 (m, 2H); 2.00-2.10 (m, 2H); 1.60-1.68 (m, 2H); 1.18 (s, 3H); 1.16 (s, 3H); 0.83 (m, 3H). Calcd for $\text{C}_{13}\text{H}_{22}\text{N}_4\text{SO}_3$: C: 49.66; H: 7.05; N: 17.82; S: 10.20. Found: C: 49.73; H: 7.15; N: 17.65; S: 10.22.

Example 17

(2S)-1-[2-(Benzotriazol-1-ylcarbonyl)pyrrolidinyl]-3,3-dimethylpentane-1,2-dione; molecular formula: $\text{C}_{13}\text{H}_{22}\text{N}_4\text{O}_3$; molecular weight: 342.39 (Compound 151).

This example was prepared according to the process of Scheme XVII as follows.



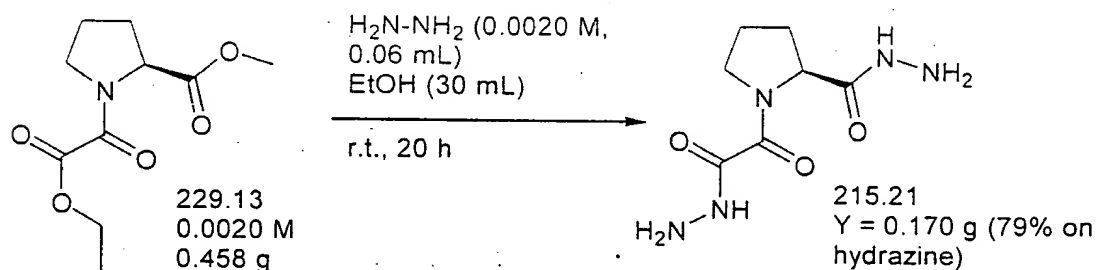
The procedure is identical to that used in the preparation of compound # 8. Crude product was purified by column chromatography (silica gel; eluent - EtOAc:hexanes 1:2).

Fractions with R_f ca. 0.50 (in EtOAc:hexanes 1:1) were collected. Evaporation of solvents gave 0.410 g of clear oil (slowly solidifies upon standing). ^1H NMR (CDCl_3 , 400 MHz): δ 8.29-8.27 (m, 1H); 8.15-8.13 (m, 1H); 7.70-7.65 (m, 1H); 7.56-7.50 (m, 1H); 6.00-5.95 (m, 1H); 3.75-2.65 (m, 2H); 2.67-2.60 (m, 1H); 2.23-2.09 (m, 3H); 1.85-1.70 (m, 2H); 1.30/1.26/1.23/1.16 (s - all 4 peaks belong to 2 rotamers, 6H); 0.89/0.76 (2 t - both belong to 2 rotamers, $J = 7.5$ Hz, 3H). Calcd for $\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_3$: C, 63.14; H, 6.48; N, 16.36. Found: C, 62.91; H, 6.44; N, 16.22.

Example 18

N-Amino-2-[2-(N-aminocarbamoyl)pyrrolidiny]-2-oxoethanamide; molecular formula: $\text{C}_7\text{H}_{13}\text{N}_5\text{O}_3$; molecular weight: 215.21 (Compound 152).

This example was prepared according to the process of Scheme XVIII as follows.

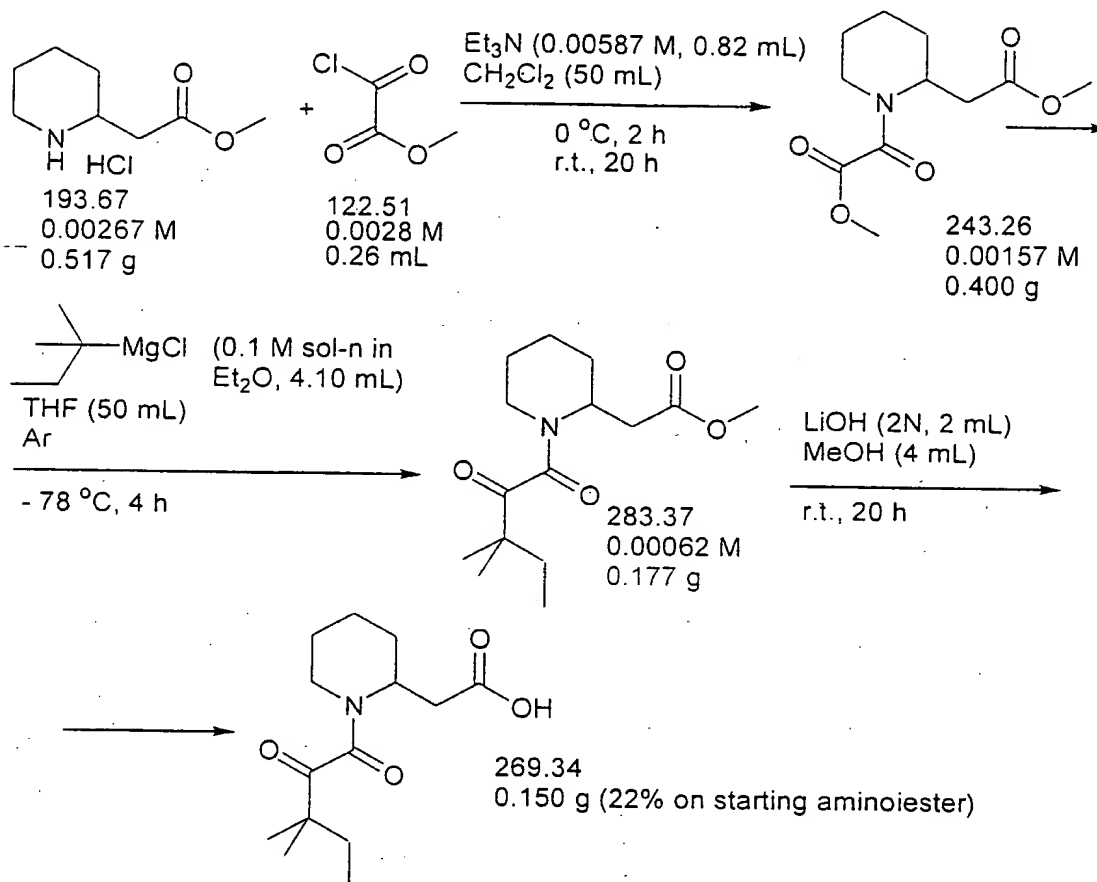


Anhydrous hydrazine (0.06 mL, 2 mmol) was added at room temperature to a stirred solution of diester (0.458 g, 2 mmol) in ethanol (30 mL). Stirring was continued for 20 h, precipitate formed was filtered, washed with diethyl ether, and air-dried. White solid (0.170 g) with m.p. $>200^\circ\text{C}$. ^1H NMR (CDCl_3 , 400 MHz): δ 9.77 (br s, 1H); 9.10/9.02 (s, 1H); 4.93-4.90/4.28-4.23 (m, 1H); 4.44/4.37 (br s, 2H); 4.17 (br s, 2H); 3.75-3.70 (m, 1H); 3.69-3.78 (m, 1H); 2.19-1.98 (m, 1H); 1.97-1.66 (m, 3H). Calcd for $\text{C}_7\text{H}_{13}\text{N}_5\text{O}_3$: C, 39.07; H, 6.09; N, 32.54. Found: C, 38.97; H, 6.10; N, 32.36.

Example 19

2-[1-(3,3-Dimethyl-2-oxopentanoyl)-2-piperidyl]acetic acid; molecular formula: $C_{14}H_{23}NO_4$; molecular weight: 269.34 (Compound 153).

5 This example was prepared according to the process of Scheme XIX as follows.



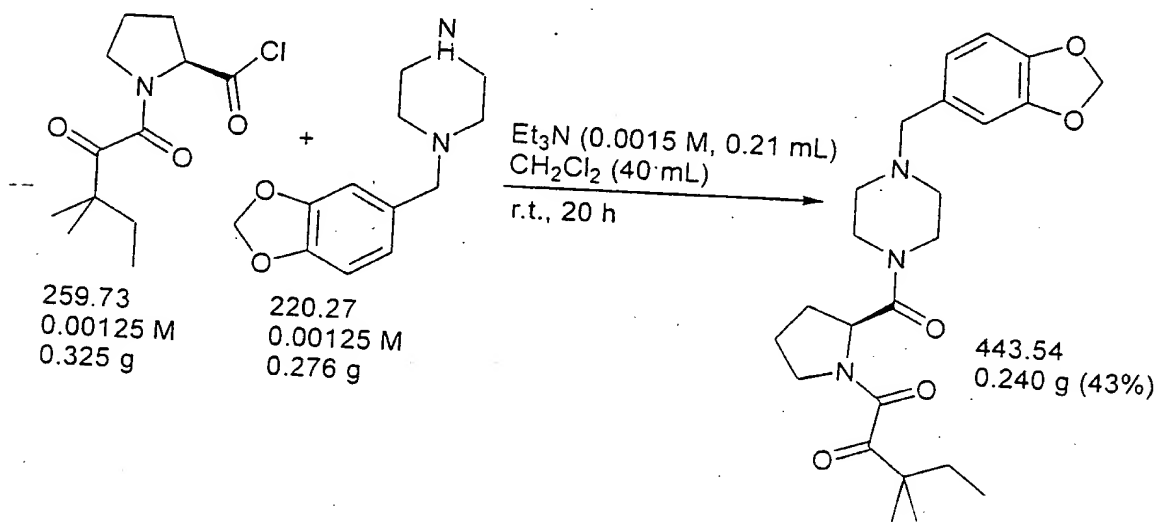
- 10 A solution of methyl chlorooxoacetate (0.26 mL, 2.80 mmol) in dichloromethane (10 mL) was added dropwise within 10 min to a stirred and chilled mixture of amino ester hydrochloride (0.517 g, 2.67 mmol) and triethylamine (0.82 mL, 5.87 mmol) in dichloromethane (40 mL). After addition
- 15 was completed, the stirring was continued at 0 °C for a total of 2 h, and at room temperature for 20 h. Water (50 mL) was added, and the mixture was transferred into separating funnel. After extraction, organic layer was separated, washed with Na_2CO_3 (sat., 15 mL), water (50 mL),

and HCl (1N, 30 mL). Organic layer was separated and dried over Na₂SO₄ (anhyd). Evaporation of the solvent *in vacuo* gave 0.405 g of light yellow oil. It was then dissolved in dry THF (50 mL) and chilled to -78 °C upon stirring. An
5 ethereäl solution of Grignard reagent (1 M, 4.10 mL) was added dropwise to the latter solution within 15-20 min, and the whole was stirred for another 3 h 45 min. A mixture was quenched by addition of NH₄Cl (sat., 25 mL) and ice-water (100g+50 mL), stirred for 5 min, and extracted
10 with diethyl ether (3x40 mL). Combined organic layers were separated and dried over MgSO₄ (anhyd). Evaporation of the solvent *in vacuo* gave 0.200 g of light oil. Crude product was purified by column chromatography (silica gel, eluent: EtOAc:hexanes, 1:1). Fractions with R_f ca. 0.55 were
15 collected; evaporation of solvents gave 0.177 g of colorless oil. (Analyzed: Calcd for C₁₅H₂₅NO₄: C, 63.58; H, 8.89; N, 4.94. Found: C, 63.79; H, 9.00; N, 4.94). The preceding oil was dissolved in MeOH (4 mL), and solution of LiOH (2N, 2 mL) was added upon stirring at room
20 temperature. Stirring was continued for 20 h. Dichloromethane (50 mL) and water (30 mL) were added, and the whole was shaken in separating funnel for 5 min. Aqueous layer was separated; organic layer was additionally washed with NaOH (1N, 25 mL), and the
25 combined aqueous layers were acidified with HCl (1N, until pH ca. 2). Dichloromethane (50 mL) was added, and the whole was shaken in separating funnel for 3 min. Organic layer was separated and dried over Na₂SO₄ (anhyd). Evaporation of the solvent *in vacuo* gave 0.150 g of clear
30 oil with R_f ca. 0.05 (EtOAc:hexanes, 4:1). ¹H NMR (CDCl₃, 400 MHz): d 5.16-5.07 and 4.41-4.46 (2m, 1H); 3.93-4.01 and 3.33-3.26 (2m, 1H); 3.17-3.08 and 2.94-2.87 (2m, 1H); 2.73-2.60 (m, 2H); 1.82-1.40 (m, 8H); 1.24, 1.21, and 1.18 (3s, 6H); 0.92-0.84 (m, 3H). Calcd for: C₁₄H₂₃NO₄: C, 62.43;
35 H, 8.61; N, 5.20. Found: C, 62.43; H, 8.64; N, 5.06.

Example 20

1-(2-([4-(2H-Benzo[3,4-d]1,3-dioxolen-5-ylmethyl) piperazinyl]carbonyl)pyrrol-idinyl)-3,3-dimethylpentane-1,2-dione; molecular formula: $C_{24}H_{33}N_3O_5$; molecular weight: 443.54 (Compound 154).

This example was prepared according to the process of Scheme XX as follows.



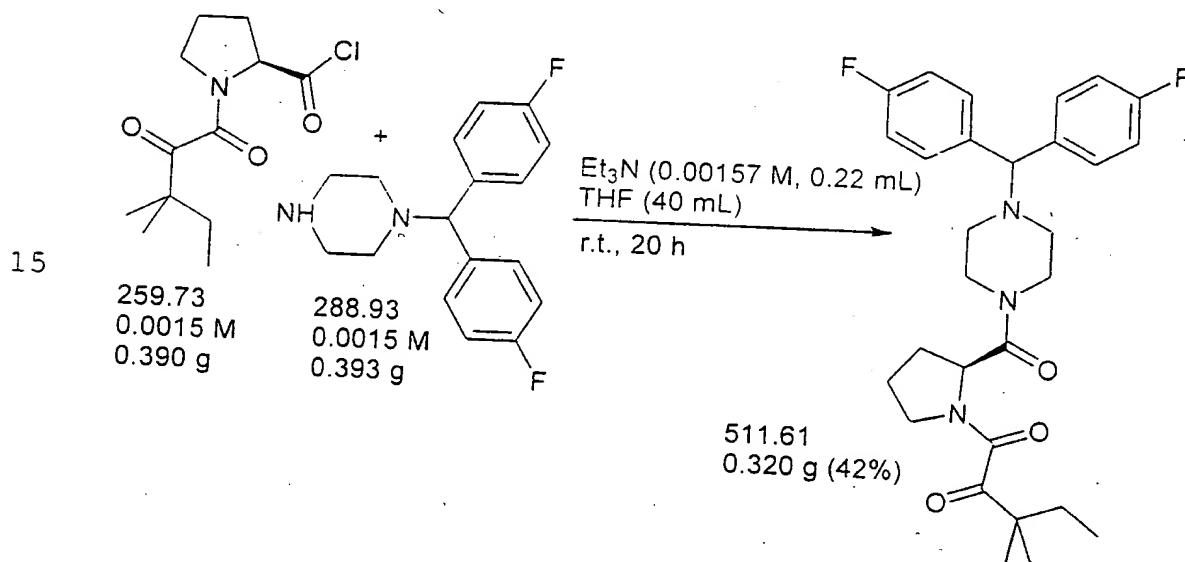
A solution of acid chloride (0.325 g, 1.25 mmol) in dichloromethane (10 mL) was added within 5 min to a stirred solution of piperazine derivative (0.276 g, 1.25 mmol) and triethylamine (0.21 mL, 1.50 mmol) in dichloromethane (30 mL) at room temperature. Stirring was continued for another 20 h. Water (50 mL) was added, and the whole was transferred into separating funnel. After extraction, organic layer was separated and washed subsequently with HCl (1N, 30 mL), NaOH 91N, 30 mL), and water (50 mL), separated again, and dried over Na₂SO₄ (anhyd). Evaporation of the solvent in vacuo gave 0.450 g of yellow oil. Crude product was purified by column chromatography (silica gel, eluent: EtOAc:hexanes, 3:1, then 4:1). Fractions with R_f ca. 0.15 (in EtOAc:hexanes, 3:1) were collected; evaporation of solvents gave 0.240 g of clear yellow oil. ¹H NMR (CDCl₃, 400 MHz): δ 6.87 and 6.85 (2s, 1H); 6.76-6.71 (m, 2H); 5.94 (s, 2H); [4.99 (dd,

J=4.6 and 8.4) and 4.86 (dd, J=3.9 and 8.4) - total for 1H]; 3.76-3.35 (m, 8H); 2.72-1.52 (m, 10H); [1.30, 1.28, 1.22, 1.12 - 4s, total for 6H]; [0.87 (t, J=7.6) and 0.80 (t, J=7.6) - total for 3H]. Calcd for $C_{24}H_{33}N_3O_5 \cdot 0.4H_2O$: C, 63.95; H, 7.56; N, 9.32. Found: C, 64.07; H, 7.48; N, 9.02.

Example 21

1-[2-((4-[Bis(4-fluorophenyl)methyl]piperazinyl)carbonyl)pyrrolidinyl]-3,3-di-methylpentane-1,2-dione; molecular formula: $C_{29}H_{35}F_2N_3O_3$; molecular weight: 511.61 (Compound 155).

This example was prepared according to the process of Scheme XXI as follows.



The procedure is identical to that used in the previous case, except the solvent (THF instead of dichloromethane). Crude product was isolated as a pink-yellow wax, which was trituated with a mixture of EtOAc:hexanes 3:2 and kept in freezer overnight. Filtration of precipitate gave off-white microcrystals (0.320 g, 42%), which were additionally purified by column chromatography (silica gel; eluent - $CHCl_3$:MeOH, 25:1). Fractions with R_f ca. 0.40 were collected. Evaporation of solvents gave 0.205 g of

white waxy solid with m.p. 58-62 °C. ¹H NMR (CDCl₃, 400 MHz): δ 8.00-7.80 (m, 4H); 7.15-7.11 (m, 4H); 4.81-4.40 (m, 4H); 4.11-4.00 (m, 1H); 3.91-3.80 (m, 1H); 3.74-3.34 (m, 4H); 3.28-3.14 (m, 1H); 2.83-2.69 (m, 1H); 2.21-2.10 (m, 2H); 2.00-1.88 (m, 2H); 1.85-1.60 (m, 4H); 1.26 and 1.22 (2s, 6H); 0.91 (t, J=7.5 Hz, 3H). Calcd for C₂₄H₃₃N₃O₅ · 1.25 H₂O: C, 65.21; H, 7.08; N, 7.87. Found: C, 65.27; H, 6.71; N, 7.73.

Example 22

A lotion comprising the following composition may be prepared.

	(%)
95% Ethanol	80.0
an N-heterocyclic carboxylic acid or carboxylic acid isostere	10.0
α-Tocopherol acetate	0.01
Ethylene oxide (40 mole) adducts of hardened castor oil	0.5
purified water	9.0
perfume and dye	q.s.

Into 95% ethanol are added an N-heterocyclic carboxylic acid or carboxylic acid isostere, α-tocopherol acetate, ethylene oxide (40 mole) adducts of hardened castor oil, perfume and a dye. The resulting mixture is stirred and dissolved, and purified water is added to the mixture to obtain a transparent liquid lotion.

5 mL of the lotion may be applied once or twice per day to a site having marked baldness or alopecia.

Example 23

A lotion comprising the following composition shown may be prepared.

	(%)
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	95% Ethanol	80.0
	an N-heterocyclic carboxylic acid or carboxylic acid isostere	0.005
	Hinokitol	0.01
5	Ethylene oxide (40 mole) adducts of hardened castor oil	0.5
	Purified water	19.0
	Perfume and dye	q.s.

Into 95% ethanol are added an N-heterocyclic carboxylic acid or carboxylic acid isostere, hinokitol, ethylene oxide (40 mole) adducts of hardened castor oil, perfume, and a dye. The resulting mixture is stirred, and purified water is added to the mixture to obtain a transparent liquid lotion.

The lotion may be applied by spraying once to 4 times per day to a site having marked baldness or alopecia.

Example 24

An emulsion may be prepared from A phase and B phase having the following compositions.

20	(A phase)	(%)
	Whale wax	0.5
	Cetanol	2.0
	Petrolatum	5.0
	Squalane	10.0
25	Polyoxyethylene (10 mole) monostearate	2.0
	Sorbitan monooleate	1.0
	an N-heterocyclic carboxylic acid or carboxylic acid isostere	0.01
	(B phase)	(%)
30	Glycerine	10.0
	Purified water	69.0
	Perfume, dye, and preservative	q.s.

The A phase and the B phase are respectively heated

and melted and maintained at 80°C. Both phases are then mixed and cooled under stirring to normal temperature to obtain an emulsion.

The emulsion may be applied by spraying once to four times per day to a site having marked baldness or alopecia.

Example 25

A cream may be prepared from A phase and B phase having the following compositions.

10	(A Phase)	(%)
	Fluid paraffin	5.0
	Cetostearyl alcohol	5.5
	Petrolatum	5.5
	Glycerine monostearate	33.0
15	Polyoxyethylene (20 mole) 2-octyldodecyl ether	3.0
	Propylparaben	0.3
	(B Phase)	(%)
20	an N-heterocyclic carboxylic acid or carboxylic acid isostere	0.8
	Glycerine	7.0
	Dipropylene glycol	20.0
	Polyethylene glycol 4000	5.0
	Sodium Hexametaphosphate	0.005
25	Purified water	44.895

The A phase is heated and melted, and maintained at 70°C. The B phase is added into the A phase and the mixture is stirred to obtain an emulsion. The emulsion is then cooled to obtain a cream.

The cream may be applied once to 4 times per day to a site having marked baldness or alopecia.

Example 26

A liquid comprising the following composition may be

prepared.

	(%)
Polyoxyethylene butyl ether	20.0
Ethanol	50.0
5 an N-heterocyclic carboxylic acid or carboxylic acid isostere	0.001
Propylene glycol	5.0
10 Polyoxyethylene hardened castor oil derivative (ethylene oxide 80 mole adducts)	0.4
Perfume	q.s.
Purified water	q.s.

Into ethanol are added polyoxypropylene butyl ether,
15 propylene glycol, polyoxyethylene hardened castor oil, an
N-heterocyclic carboxylic acid or carboxylic acid
isostere, and perfume. The resulting mixture is stirred,
and purified water is added to the mixture to obtain a
liquid.

20 The liquid may be applied once to 4 times per day to
a site having marked baldness or alopecia.

Example 27

A shampoo comprising the following composition may be
prepared.

	(%)
Sodium laurylsulfate	5.0
Triethanolamine laurylsulfate	5.0
Betaine lauryldimethylaminoacetate	6.0
Ethylene glycol distearate	2.0
30 Polyethylene glycol	5.0
an N-heterocyclic carboxylic acid or carboxylic acid isostere	5.0
Ethanol	2.0
Perfume	0.3

Purified water	69.7
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Into 69.7 of purified water are added 5.0 g of sodium laurylsulfate, 5.0 g of triethanolamine laurylsulfate, 6.0 g of betaine lauryldimethyl-aminoacetate. Then a mixture obtained by adding 5.0 g of an N-heterocyclic carboxylic acid or carboxylic acid isostere, 5.0 g of polyethylene glycol, and 2.0 g of ethylene glycol distearate to 2.0 g of ethanol, followed by stirring, and 0.3 g of perfume are successively added. The resulting mixture is heated and subsequently cooled to obtain a shampoo.

The shampoo may be used on the scalp once or twice per day.

Example 28

A patient is suffering from alopecia senilis. An N-heterocyclic carboxylic acid or carboxylic acid isostere, or a pharmaceutical composition comprising the same, may be administered to the patient. Increased hair growth is expected to occur following treatment.

Example 29

A patient is suffering from male pattern alopecia. An N-heterocyclic carboxylic acid or carboxylic acid isostere, or a pharmaceutical composition comprising the same may be administered to the patient. Increased hair growth is expected to occur following treatment.

Example 30

A patient is suffering from alopecia areata. An N-heterocyclic carboxylic acid or carboxylic acid isostere, or a pharmaceutical composition comprising the same, may be administered to the patient. Increased hair growth is expected to occur following treatment.

Example 31

A patient is suffering from hair loss caused by skin lesions. An N-heterocyclic carboxylic acid or carboxylic acid isostere, or a pharmaceutical composition comprising the

same, may be administered to the patient. Increased hair growth is expected to occur following treatment.

Example 32

5 A patient is suffering from hair loss caused by tumors. An N-heterocyclic carboxylic acid or carboxylic acid isostere, or a pharmaceutical composition comprising the same, may be administered to the patient. Increased hair growth is expected to occur following treatment.

Example 33

10 A patient is suffering from hair loss caused by a systematic disorder, such as a nutritional disorder or an internal secretion disorder. An N-heterocyclic carboxylic acid or carboxylic acid isostere, or a pharmaceutical composition comprising the same, may be administered to the patient. Increased hair growth is expected to occur following treatment.

Example 34

20 A patient is suffering from hair loss caused by chemotherapy. An N-heterocyclic carboxylic acid or carboxylic acid isostere, or a pharmaceutical composition comprising the same, may be administered to the patient. Increased hair growth is expected to occur following treatment.

25 Example 35

A patient is suffering from hair loss caused by radiation. An N-heterocyclic carboxylic acid or carboxylic acid isostere, or a pharmaceutical composition comprising the same may, be administered to the patient. Increased hair growth is expected to occur following treatment.

Example 36

A patient is suffering from a neurodegenerative disease. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or a pharmaceutical composition comprising

the same is administered. It would be expected that the patient would improve their condition or recover.

Example 37

5 A patient is suffering from a neurological disorder. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or pharmaceutical compositions comprising same is administered. It would be expected that the patient would improve their condition or recover.

Example 38

10 A patient is suffering from stroke. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or pharmaceutical compositions comprising same is administered. It would be expected that the patient would improve their condition or recover.

15 Example 39

A patient is suffering from Parkinson's Disease. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or pharmaceutical compositions comprising same is administered. It would be expected that the patient would improve their condition or recover.

Example 40

25 A patient is suffering from Alzheimer's Disease. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or pharmaceutical compositions comprising same is administered. It would be expected that the patient would improve their condition or recover.

Example 41

30 A patient is suffering from Huntington's Disease. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or pharmaceutical compositions comprising same is administered. It would be expected that the patient would improve their condition or recover.

Example 42

A patient is suffering from a peripheral neuropathy. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or pharmaceutical compositions comprising same is administered. It would be expected that the patient
5 would improve their condition or recover.

Example 43

A patient is suffering from amyotrophic lateral sclerosis. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or pharmaceutical compositions
10 comprising same is administered. It would be expected that the patient would improve their condition or recover.

Example 44

A patient is suffering from a spinal injury. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or pharmaceutical compositions comprising
15 same is administered. It would be expected that the patient would improve their condition or recover.

Example 45

A patient is at risk of suffering from a neurodegenerative disease or neurological disorder. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring or a pharmaceutical composition comprising
20 the same is prophelactically administered. It would be expected that the patient would be prevented from some or all of the effects of the disease or disorder, or would significantly improve their condition or recover over patients
25 who were not pre-treated.

Example 46

A patient is suffering from macular degeneration. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other neopxic factors, or a pharmaceutical composition comprising the same, may be
30

administered to the patient. A reduction in vision loss, prevention of vision degeneration, and/or promotion of vision regeneration are/is expected to occur following treatment.

Example 47

5 A patient is suffering from glaucoma, resulting in cupping of the optic nerve disc and damage to nerve fibers. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other neopxic factors, or a
10 pharmaceutical composition comprising the same, may be administered to the patient. A reduction in vision loss, prevention of vision degeneration, and/or promotion of vision regeneration are/is expected to occur following treatment.

Example 48

15 A patient is suffering from cataracts requiring surgery. Following surgery, a carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other neopxic factors, or a pharmaceutical composition comprising the same, may be
20 administered to the patient. A reduction in vision loss, prevention of vision degeneration, and/or promotion of vision regeneration are/is expected to occur following treatment.

Example 49

25 A patient is suffering from an impairment or blockage of retinal blood supply relating to diabetic retinopathy, ischemic optic neuropathy, or retinal artery or vein blockage. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other neopxic factors, or a
30 pharmaceutical composition comprising the same, may be administered to the patient. A reduction in vision loss, prevention of vision degeneration, and/or promotion of vision regeneration are/is expected to occur following treatment.

Example 50

35 A patient is suffering from a detached retina. A

carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other neoplastic factors, or a pharmaceutical composition comprising the same, may be administered to the patient. A reduction in vision loss, prevention of vision degeneration, and/or promotion of vision regeneration are/is expected to occur following treatment.

Example 51

A patient is suffering from tissue damage caused by inflammation associated with uveitis or conjunctivitis. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other neoplastic factors, or a pharmaceutical composition comprising the same, may be administered to the patient. A reduction in vision loss, prevention of vision degeneration, and/or promotion of vision regeneration are/is expected to occur following treatment.

Example 52

A patient is suffering from photoreceptor damage caused by chronic or acute exposure to ultraviolet light. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other neoplastic factors, or a pharmaceutical composition comprising the same, may be administered to the patient. A reduction in vision loss, prevention of vision degeneration, and/or promotion of vision regeneration are/is expected to occur following treatment.

Example 53

A patient is suffering from optic neuritis. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other neoplastic factors, or a pharmaceutical composition comprising the same, may be administered to the patient. A reduction in vision loss, prevention of vision degeneration, and/or promotion of vision

regeneration are/is expected to occur following treatment.

Example 54

5 A patient is suffering from tissue damage associated with a "dry eye" disorder. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other neoplastic factors, or a pharmaceutical composition comprising the same, may be administered to the patient. A reduction in vision loss, prevention of vision degeneration, and/or promotion of vision regeneration are/is expected to occur following treatment.

Example 55

15 A patient is suffering from sensorineural hearing loss. A carboxylic acid or carboxylic acid isostere of an N-heterocyclic ring as identified above, alone or in combination with one or more other factors, or a pharmaceutical composition comprising the same, may be administered to the patient. A reduction in hearing loss is expected to occur following treatment.

20 The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the following claims.

25